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The Naval Medical Research Institute

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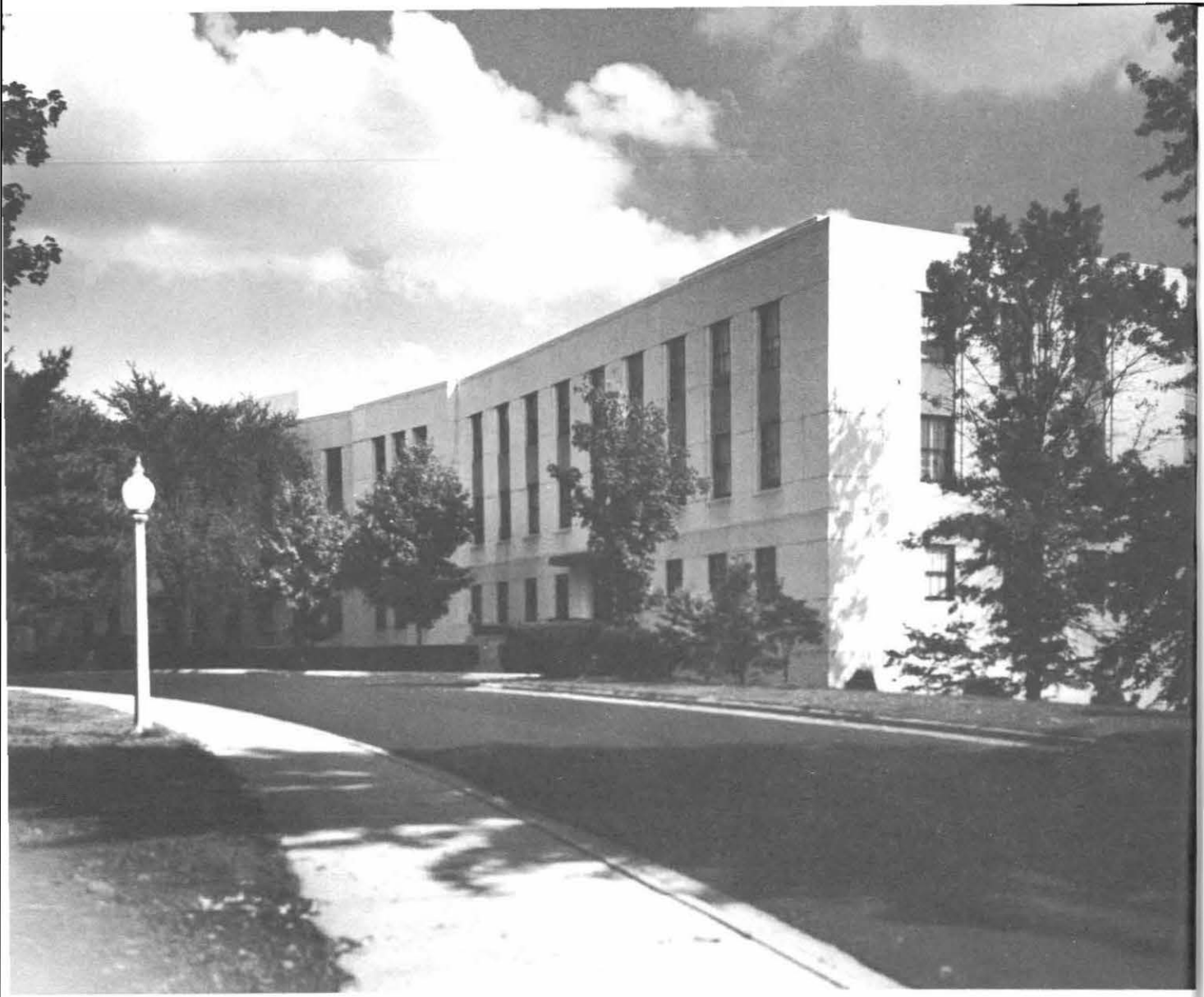

The Naval Medica

Edited by
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Research Institute

1942–1962

FOREWORD

THE FIRST 20 YEARS of the Naval Medical Research Institute's existence encompassed most of World War II, the Korean conflict, the rebirth of "limited" warfare and "counterinsurgency" as important military operations, the advent of nuclear weapons and power, the beginnings of exploitation of space, and the regained importance of undersea operations to the Navy. Major advances in technology along with a rapidly changing socioeconomic and political world wrought major changes in the naval mission, strategy, weaponry, and manpower needs with attendant changes in medical requirements.

This 20 years has also seen vast changes in medicine. As government and industry have expanded research effort, a concentration of talent has been brought to bear on many disease problems and has resulted in a weltering array of new diagnostic, surgical, and therapeutic techniques leading to ever greater specialization and requirements for the modern hospital complex to provide the care which an informed society now expects. Basic biomedical research promises better understanding of diseases and means for their prevention yet prevention lags far behind therapy in both the research effort and application.

Twenty years is also a generation in the history of man. Those scientists of the prewar and World War II era who played such a large role in shaping the research effort at the Naval Medical Research Institute are being replaced by a younger generation which looks to the future. Since the value of a research institution can only be measured by its past record, the reputation it has established, and the contributions it has made, it has seemed worthwhile to have some of the major research efforts at the Institute reviewed and summarized by those most familiar with the efforts. This record of accomplishments and failures, of contributions to the Navy and to medicine in general, will thus provide a base upon which a continuing tradition of service to the Navy and their country can be built by those who follow.

JOHN R. SEAL
Captain, MC, USN
Commanding Officer

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I. INTRODUCTION

D. E. Goldman

The material presented in this volume represents an attempt to provide the scientific and military communities with a panoramic view of the research activities of the Naval Medical Research Institute (NMRI) over its first 20 years of existence. These activities have dealt with a wide variety of basic and applied problems and have been related to many facets of naval medicine. They have involved many scientific, clinical, and technical areas and have also involved collaboration with other laboratories, field installations, and fleet units. Complete coverage of the work done is impossible, partly because over the years there have been changes in emphasis and turnover of personnel and partly because of the difficulty in following a problem from its laboratory aspects through to its application in the field. Some areas of research have, therefore, been reviewed in some detail; others have been chronicled and still others merely listed. Certain areas have had to be omitted for one reason or another. The material is largely in the form of scientific reviews, but distributed throughout are examples of applied research and of the development of procedures for preventive and therapeutic medicine in relation to field and hospital situations.

A short historical note is in order. The Naval Medical Research Institute was commissioned in October 1942 with an initial staff of 13 officers and 50 enlisted men. The staff grew to about 200 as wartime activities expanded and was at the time almost entirely military. The work was largely concerned with the development and testing of devices and methods to be used by the fleet. Extensive studies were carried out on protective clothing, desalination of sea water, aviation oxygen equipment, insect repellents and other means of insect control, physiological effects of tropical environment, and the effects of immersion in cold water. Vaccines, night vision, body armor, nutrition, oral hygiene, tropical diseases, and parasites were investigated. Shortly after the end of the war, NMRI participated in the Bikini atomic bomb tests and has since contributed largely to the knowledge of the biological effects of radiation. Growth in this field eventually led to the formation of a new laboratory (Armed Forces Radiobiology Research Institute) to handle some of the applications of radiobiology.

In 1945 the physical plant was approximately doubled in size and later a modern completely equipped animal laboratory was added. Another section was added to the laboratory in 1957.

After the end of World War II there was a rapid shrinkage in the size of the Armed Forces and many of the investigators returned to civilian life. Much of the Institute's program, however, was retained although with a shift in emphasis from development and testing to research. A civilian component was gradually added to the staff and in recent years, the total staff has remained more or less evenly distributed between military and civil service personnel, with an average of 40 to 50 at the professional level.

The function of an active medical research laboratory in a military organization warrants some discussion. Among the many problems faced by the Navy is the maintenance of the health and effectiveness of its personnel. Traditionally, the Medical Department has cared for the sick and wounded and has advised on ways and means of avoiding illness. As the scope and complexity of military operations have continued to expand, the extent and complexity of the health problems have increased to the point where specialized knowledge is now required to solve them. Thus, the need for research has continued to increase and there are now a number of active laboratories within the

Medical Department whose primary mission is the solving of as many of these health problems as time, skill, and facilities will permit. Some of these problems are common to all mankind, or at least to such of mankind as may happen to be involved in certain situations. Some of the problems are either peculiar to Navy operations or are so likely to occur in Navy operations that the Navy must deal with them independently of any general interest and importance which they may have.

Of the several research installations of the Medical Department, the Naval Medical Research Institute has the most broad interests and deals with the most basic aspects of naval medical problems. One of its persistent activities is, in fact, the investigation of those research areas which underlie most directly the practical problems faced in naval medicine. These problems are at least as much preventive as therapeutic. Although the traditional activity of the medical profession has been in the area of cure, it is obviously more efficient to prevent when at all possible. In any case, both prevention and cure require an understanding of cause, and the search for this understanding is what is often meant by basic research. The results of basic research can then be used to provide practical means for the solution of important problems. Progress is through a stage of applied research in which specific information is sought, to development work where the end product may be a device but in a medical context is most often a procedure or set of working rules.

To accomplish its mission the Naval Medical Research Institute requires trained scientists and physicians, people who not only carry out competent research but who also are able to relate their research to Navy problems. The overall process is not a direct or simple one. Rarely is it possible to analyze a problem, carry out the needed research, and apply it to the field problem all in one sequential chain. Biomedical problems have a stubborn habit of being complex and interdisciplinary. It is characteristic of research activities that the results of one study may be very useful in an area outside that of the worker's immediate interest. For example, work on muscle cells has proved to be valuable in the study of fatigue. Research on the neurophysiology of the brain stem may help solve difficult problems in submarine medicine. Examples could be multiplied. What is important is that, especially in biomedical work, the extensive interactions of scientific activities belie the notion that mission oriented research must be directly related to the end product.

Proper analysis of a problem involves expert judgment as to the selection of an approach, effectiveness of equipment, evaluation of data, and often the education of the consumer in the use of the solution. Clearly, high caliber personnel are needed: imaginative, forceful, and expert. The accommodation of such people in a military laboratory requires maximum use of their scientific judgment and in order to maintain such an expert staff, personnel turnover must be kept at a low level. It is totally impractical to hire people for specific duties, let them go at the termination of a particular study, and then try to find new people for new work. The best procedure which has been found is to select highly competent and imaginative scientists whose interests lie within the major fields of interest of the Navy, to support them, and to encourage their cooperation. To the extent that they respond by turning out useful work in the general areas of relevance, the formula is a success. It applies equally whether the scientists are civilian or military.

In the chapters which follow, a number of the more active areas of the Institute's work are covered in greater or lesser detail. At the end of the volume is a bibliography of the Institute's publications which is believed to be nearly complete. Those who are interested in the Institute's work will find a perusal of that bibliography illuminating. Considerable work has been done, for example, in areas such as submarine medicine, including the preparation of decompression tables, tolerance to atmospheric carbon dioxide, oxygen and nitrogen toxicity. In the area of shipboard medicine, as such, one will find references to work on motion sickness, protection against sunburn, and problems in hygiene and toxicology. A particularly active area has been that of experimental surgery, in which there has been considerable work related to the development of open heart surgery and vascular prostheses. This research has also contributed to the establishment of a tissue bank at the naval hospital in Bethesda. Nearly 400 senior authors have been associated with the preparation of approximately 1,800 reports ranging all the way from short technical notes through extensive research reports and reviews. Much of the material has appeared in the scientific literature.

No apology is made for the obvious gaps in coverage of work in the summaries which follow. No one individual or group has had sufficient continuity to have complete perspective of the problems and of NMRI's contributions in problem areas. The failure to develop senior scientists with such perspective will be particularly noted in such areas as underwater physiology and clinically oriented research. It is in such areas that the bibliography will be most useful.

II. HOT AND COLD ENVIRONMENTS

David Minard

Introduction

Man is a homeothermic animal. Under conditions of excessive environmental heat he maintains a normal body temperature by secreting and evaporating sweat on his body surface. Evaporation of each gram of sweat dissipates 580 gram-calories of heat. Under extremely hot conditions evaporation of as much as a liter of sweat each hour may be required to maintain heat balance between his body and the environment.

In cold environments, on the other hand, excessive loss of heat from the body results in shivering, which is an involuntary form of muscular work. Work of any form tends to raise the rate of body heat production and thus reduces discomfort of a cold environment by offsetting losses of body heat. In a hot environment, hard work increases the heat load on the physiological mechanism of temperature regulation. In military operations, particularly in combat, men are exposed to wider extremes of temperature, for longer periods, both in natural as well as in artificial environments, than in industrial occupations or other peacetime pursuits.

Thermal Injury

Failure of the body to compensate for either external heating by hot environments or internal heating from elevated heat production by work or by a combination of these two factors leads to heat illness which may take one of three major forms: (*a*) heat exhaustion which results from circulatory deficiency, (*b*) heat cramps which results from depletion of body salt, or (*c*) heat stroke which results from failure of the body to form sweat. Heat stroke is the most serious form of heat illness because without benefit of evaporative cooling, the body rapidly stores heat. Unless the victim is promptly and effectively cooled the high internal temperature damages his brain and other vital tissues with death as a frequent result.

Exposure of unacclimatized troops to hot desert and tropical climates such as occurred in Allied forces operating in the Middle East, in Southeast Asia, in the Philippines, and in New Guinea in World War II led to high incidence rates of heat casualties which at times exceeded those resulting from enemy action. More recently the deleterious effects of environmental heat were experienced by American combat units who were landed in Lebanon in July 1958. In the summer of 1961 British units airlifted from the United Kingdom into Kuwait were suddenly exposed to the extreme heat of the Persian Gulf area. According to an article in the London Times published in July 1962, the incidence rate of heat casualties in these unacclimatized units was about 10 percent. This article referred to the opinion of an expert on heat stress who estimated that the rate of heat casualties in these units would have been 50 percent had they been committed immediately to combat.

The monthly incidence of heat casualties in the Navy and Marine Corps of the United States reaches a peak in June, indicating that military personnel are more susceptible to heat effects early in the summer season before they have acquired full acclimatization. The Marine Corps experiences a higher incidence of heat casualties than the Navy. Relatively high rates of casualties in Marines occur during combat exercises, particularly those conducted in early spring when units of the 3d Marine Division from Okinawa are landed in training areas in the Philippine Islands. The change in climate is abrupt. In March 1962, approximately 75 moderate to serious heat casualties

with 1 death from heat stroke occurred in a single Marine battalion during the first day of a 5-day exercise on the island of Mindoro.

Under conditions of extreme cold, increased heat production from voluntary work or shivering may be insufficient to maintain deep body temperature and the temperature of vital tissues at the body core will begin to fall. This is known as general hypothermia. Fatal levels of hypothermia can develop within an hour or less as a result of accidental immersion in sea water at temperatures of 28° to 30° F. which are encountered at high latitudes. A large majority of deaths from immersion which occurred in survivors of Allied ships sunk in the Atlantic by enemy action in World War II can be attributed to hypothermia, rather than drowning because in water temperatures as high as 70° F., survival time is measured in hours rather than in days. Even when the survivor succeeds in reaching a raft or an open lifeboat, low sea and air temperatures are important factors contributing to mortality. On exposure to air at subfreezing temperatures when clothing protection is inadequate, hypothermia may also occur but generally develops more slowly than in immersion hypothermia. Under these conditions local cold injury usually precedes general hypothermia and takes the form of frostbite. With air temperatures somewhat above 32° F. a more slowly developing form of cold injury can occur which is known as immersion foot or trench foot. The sequellae of nonfreezing cold injury, however, may be more disabling than frostbite. It is well known that cold was the major factor in the defeat of Napoleon's army in his Russian campaign and that cold injury took a heavy toll in both the Allied as well as the enemy forces in both World Wars. In World War II the American Army in Europe sustained over 55,000 casualties from cold. Over 5,000 casualties were reported among United Nations units in Korea in the winter of 1950–51.

Effect of Thermal Stress on Performance

Individual tolerance to extreme temperatures varies widely. Morphological factors of body habitus, biochemical factors related to body composition and nutrition, physiological factors which underlie acclimatization and physical fitness, and other factors including adequate clothing, indoctrination, training, and leadership all play important roles in determining the degree to which thermal stress will adversely affect performance of units in military operations. In a group of healthy young men in military service exposed to the same hot or cold climate, only a small percentage may become actual casualties in the clinical sense. However, the incidence of such casualties, although constituting an important medical problem, should be considered significant chiefly because it serves to indicate prevailing conditions of heat or cold severe enough to hamper effective performance by the military unit as a whole. It has been clearly established that there is deterioration both in quality and quantity of work performed during exposure to extreme temperatures. Certain skilled tasks requiring high degrees of muscle coordination and purely mental tasks such as those involved in deciphering a code are adversely affected by relatively mild degrees of heat stress.

In severe cold, numbness of the hands and fingers interferes seriously with tasks involving manuel dexterity. The weight and bulk of clothing needed for insulation against cold further reduces efficiency of performance. Problems of nutrition, environmental sanitation, and the medical care of casualties are all greatly magnified in military operations conducted in frigid zones. At temperatures below −50° F. the effectiveness of men, vehicles, and weapons approaches zero.

The present aim of naval medical research on thermal factors in the environment, therefore, is not merely to establish limits of physiological tolerance to thermal stress but more importantly to define environmental conditions and to develop operational practices which will promote maximum efficiency and optimum health in Navy and Marine Corps personnel whose duties require brief or prolonged exposure to extreme temperatures.

Research on Thermal Stress Problems

Shipboard standards for environmental temperature and humidity.—From 1943 through 1948 a number of reports were issued by the Naval Medical Research Institute dealing with adverse effects of heat stress on comfort, health, and efficiency of Navy personnel (131, 428, 901, 1153, 1216, 1222, 1223, 1786). These studies were conducted not only in laboratory hot rooms at NMRI but also aboard combat vessels and hospital ships cruising in tropical waters. Thermal standards for comfort and for maximum tolerable heat stress established by these reports appear in the Manual of Naval Preventive Medicine (ch. 3) and serve as a guide to engineers of BuShips in designing ventilating and cooling systems for new construction and for revision of existing shipboard installations.

Heat stress in machinery spaces during ventilation shutdown.—When under attack by nuclear, biological, or chemical weapons, Navy surface vessels must "button-up" to prevent contamination of the ship's interior. Studies conducted by NMRI in 1951 indicated that ventilation shutdown leads to rapid accumulation of intolerably high levels of humid heat in machinery spaces (1786). As one consequence of these findings, steam propelled Navy ships are now designed with mechanically cooled console rooms within the machinery space from which propulsion equipment can be remotely controlled during emergency ventilation shutdown. Individually cooled suits are being developed for engine room personnel whose duties demand their exposure to intense heat stress in unventilated areas of the machinery space.

Heat stress in machinery spaces and laundry.—Mechanical cooling of spaces accommodating the ship's engines and boilers as well as its laundry is not feasible because the weight and bulk of cooling equipment needed would be prohibitive. Heat stress on men at work stations in these areas is alleviated by "spot cooling," a system in which outside air is directed on men at high velocity from a supply terminal. Propulsion equipment of new ships is now designed to operate at approximately twice the steam pressure of older ones. It was necessary, therefore, to reexamine existing standards for ventilation and to provide engineers with more valid criteria for designing these systems.

In 1958 observers from BuShips and from NMRI made simultaneous physical measurements on thermal conditions and physiological measurements on personnel on watch in the machinery and laundry space of a large new aircraft carrier during flight operations in the Caribbean (1133). As a result of these studies, improvement in engineering control of heat stress in these working spaces was initiated. An unexpected finding was that certain well known heat stress indices based on computations involving temperature, humidity, air velocity, and radiant heat proved of little value in predicting heat effects on the men. This indicated the need for further research on heat stress under conditions more closely simulating the complex thermal environment found in machinery spaces of ships.

Impermeable protective clothing.—In January 1962 the Bureau of Ships requested NMRI to determine limiting conditions of air temperature under which men exposed on weather decks of ships to enemy attack by biological and chemical agents could operate effectively for at least 4 hours while wearing protective clothing consisting of external impermeable garments, gas mask, rubber boots and gloves, worn over a chemically impregnated suit. The aim was not to design new garments but rather to utilize gear already on board Navy ships.

These studies are still in progress but one important finding is that a continuous water spray directed on the subject raises the limiting ambient air temperature for working for 4 hours in this assembly from less than 75° F. under dry conditions to 95° F. when cooled by spray. Shipboard tests have also been conducted.

Other forms of protective equipment such as body armor also interfere with dissipation of body heat in hot climates. Effects of body armor and load carrying equipment on combat effectiveness is a subject which has been under active study at the Naval Medical Field Research Laboratory at the Marine Corps Base, Camp Lejeune, N.C.

Prevention of heat casualties in recruits.—Unacclimatized recruits exposed to heat stress during summer training experience a high rate of heat casualties. The incidence rate in Marine Corps recruits at Parris Island, S.C., has been significantly reduced since the introduction in 1956 of a program which curtails strenuous training exercises during spells of hot weather (1134, 1786, 1787). Heat stress is evaluated in terms of the Wet Bulb-Globe Temperature Index of Yaglou which is based on hourly readings of the wet bulb, dry bulb, and black globe thermometers weighted as follows:

$$0.7\ WB + 0.1\ DB + 0.2\ GT = WBGT\ Index.$$

At index levels of 85° F. and above vigorous training is curtailed for new recruits. More seasoned recruits continue regular training until the index reaches 88° F. or above. Other key elements in the program are: emphasis on ample water and salt intake; rational clothing practices, conditioning platoons for obese recruits; a breaking-in period for all recruits; indoctrination of recruits and instructors in basic elements of hot weather hygiene; and perhaps most importantly, active support by the training command.

Lack of acclimatization in personnel engaged in combat operations.—When suddenly transported from a cool climate to a hot climate in other parts of the world, highly trained troops suffer the impact of environmental heat stress because they are not adequately acclimatized. The cost to the military operation is twofold: first, loss of personnel who become heat casualties; and secondly, reduced combat effectiveness

of the unit as a whole. The latter is the more important.

On February 19, 1959, a battle group of Army paratroopers from Fort Bragg, N.C., was airlifted to a drop zone near the Rio Hato, Republic of Panama, where they parachuted and reassembled. Immediately thereafter they began a 2-day combat exercise against an "agressor" force made up of Army units stationed in the Canal Zone. Body weight, heart rate, and body temperature, as well as urinary volume, specific gravity, and electrolytes were recorded at 1700 on each day of the exercise and compared with measurements made before the exercise and after return to Fort Bragg (1135).

All test measurements differed significantly from the control and recovery measurements. The pattern disclosed was that of moderate to severe dehydration associated with salt imbalance, disturbances in thermoregulation, and circulatory strain. Line and medical officers observing the exercise stated that combat efficiency of the units from Fort Bragg was seriously impaired. By contrast, a control group of 10 "aggressors" who had been stationed in the Panama area for at least 6 months showed no significant deviation from normal in these measurements, nor was a performance decrement noted. In 1960 and 1962 these findings were confirmed and extended in careful and well-controlled investigations conducted in Aden by the British War Office. These field studies demonstrate once again the serious physiological handicap under which unacclimatized foot soldiers must operate when suddenly transported from a cool to a hot climate. Methods for increasing heat tolerance of unacclimatized men before transfer to hot climates are urgently needed.

Local cold injury.—Fundamental studies on the physiological-pathology underlying immersion foot were contained in several reports from NMRI which appeared during and immediately following World War II (1218, 1451, 1452, 1453, 1454). Report No. 3 of this series describes in detail the vapor barrier principle subsequently adopted in the now famous combat boot which proved so successful in the Korean conflict.

Experimental frostbite is currently under study at NMRI. By use of carefully standardized methods of freezing and thawing of extremities in experimental animals it has been possible first to confirm the tissue-saving benefit of rapid rewarming, and more recently to provide supporting evidence for the additional advantage obtained by immediate sympathectomy (687).

Hypothermia.—Body cooling during water immersion and the design of immersion and exposure suits were subjects actively investigated at NMRI during and following World War II (1027, 1030, 1200, 1201, 1456, 1457, 1459, 1460, 1780). Field tests at Argentia Bay in March 1951, demonstrated that men in ordinary clothing can abandon ship by jumping into water at 37° F., swim to an inflated lifeboat equipped with canopy and sleeve openings, and there survive for 5 days on a daily ration of 100 grams of carbohydrate and 400 ml of water (1127). Current studies at NMRI are being conducted on physiologic effects of immersion under moderately cool conditions.

Laboratory studies using the human gradient calorimeter.—The human gradient calorimeter at NMRI is an important new laboratory tool for studying reactions of the resting or working human subject to heat and cold. It differs from classical calorimeters in that its response to changes in heat output is rapid, linear, and both sensitive and accurate. Temperature conditions within the calorimeter can be controlled from near freezing to temperatures well above human heat tolerance. Heat output is recorded continuously and automatically, with the capability of recording the major partitions separately, as well as the total output. A detailed discussion of this device along with other applications is given in chapter VI. To date studies utilizing the human calorimeter have led to modifications in the classical concepts of body temperature regulation. It will also contribute to solution of problems of immediate practical interest to field medicine. A few examples can be cited: (*a*) Selection and screening of personnel whose assignments require exposure to extreme temperatures, (*b*) Development of procedures for rapid acclimatization to heat or cold, (*c*) Evaluation of protecting clothing, (*d*) Evaluation of heat loads imposed by protective equipment such as body armor, and (*e*) Selection of the proper interval and duration for rest periods to prevent cumulative effects of thermal stress and fatigue in personnel working at extreme temperatures.

III. RADIATION BIOLOGY

E. P. Cronkite

Ionizing radiation and its effect upon men and materials has developed into a major military problem. Radiation became a casualty producing agent with the introduction of nuclear weapons. Radiation is produced at the time of the explosion and continues with decreasing intensity from the residual radioactive materials and from neutron induced radioactivity. Rainout and fallout from atmospherically borne radioactive debris may contaminate huge land masses with psychologically annoying or lethal levels of ionizing radiation to human beings. Similarly ships and aircraft may become annoyingly or dangerously contaminated by operation in and through contaminated atmospheres or waters. For the Navy, radiation problems are not limited to the actual use of nuclear weapons in warfare. The development of ship propulsion by nuclear reactor generated power is spreading throughout the fleet. From nuclear weapons and the use of nuclear reactors for power, naval personnel may be exposed to small doses of gamma rays and neutrons at all energies over long periods of time. Reactor accidents although highly unlikely may expose naval personnel to high doses over a short period. A problem unique to the Navy is the concentration of certain fission products in marine life growing on the ships' bottoms. In peacetime maneuvers and training there is a hazard from the components of nuclear reactors, radiation from spent fuel rods, leaks in nuclear weapons systems (tritium gas), accidental nonnuclear explosions of nuclear weapons systems (contamination of wounds, inhalation or ingestion of plutonium and uranium). These are just a few of the potential radiation problems with which the Navy of today is confronted. Exposure to radiation may result in acute injury or may produce injury that remains hidden in the information system of proliferating cells that is expressed years later as leukemia, diminution in longevity, cataracts, etc.

The understanding of ionizing radiation in all its forms and knowledge of its effects on living things commenced less than three score and ten years ago. In 1895 Roentgen discovered X-rays. In 1900 Kassabian commenced the description of radiation dermatitis of his hands that would later result in radiation cancer followed by amputations and ultimately his death in 1909. In 1905 Einstein formulated the theory of relativity which laid the theoretical background for the ultimate development of nuclear energy. In the period 1895 through 1940 there was intensive study of radiation effects on living tissue. These were pursued vigorously, particularly in France and Germany initially, and later England, the United States, and elsewhere. Of particular note are the pathologic and hematologic studies of Heinecke, Lacassagne, Fabricius-Møller (Denmark), and many others which form the foundation upon which qualitative pathologic and hematologic effects of radiation are still based. In this period, however, studies were primarily focused upon local irradiation and were generated from the desire to improve radiation therapy of cancer in addition to obtaining a basic understanding of the mechanism of the effects of ionizing radiation upon tissue. In 1932 the introduction of the roentgen unit as an adequate measure of the radiation delivered in air brought radiation biology to the level of a quantitative science. The neutron was discovered by Chadwick in 1932. Nuclear fission of uranium was observed by Hahn and associates in 1939. In this year Einstein communicated to President Roosevelt the ideas of nuclear physicists upon the military possibilities of atomic energy and bombs. Government support of fundamental studies on nuclear fission was

then initiated. Basic studies on uranium fission, neutron moderation and reactor theory culminated in successful operation of sustained fission in first pile by Fermi and associates in December of 1941. In September 1942, the Manhattan Engineering District was formed to produce the required amounts of fissionable material for the development of an atomic bomb. Full cooperation of American and British scientific communities was obtained along with that of other noted physicists. On July 16, 1945, the first atomic bomb was exploded experimentally at Alamogordo, N. Mex. On August 6, 1945, the first military use was made of an atomic bomb in an attack on Hiroshima and August 9, 1945, the second atomic bomb was used at Nagasaki.

A few days thereafter the Japanese capitulated unconditionally. The United States' strategic bombing survey groups extended their activities to Japan. Since nuclear weapons brought a new magnitude of destruction to warfare, the final conclusions of the strategic bombing survey are pertinent. "To avoid destruction, the surest way is to avoid war." This was the survey's recommendation after viewing the rubble of German cities and it holds equally true whether one remembers the ashes of Hiroshima or considers the vulnerability of American cities. "Our national policy has consistently had as one of its basic principles the maintenance of peace. Based on our ideals of justice and of peaceful development of our resources, this disinterested policy has been reinforced by a clear lack of anything to gain from war—even in victory. No more forceful arguments for peace and for international machinery of peace than the sight of the devastation of Hiroshima and Nagasaki have ever been devised. As the developer and exploiter of this ominous weapon, our nation has a responsibility, which no American should shirk, to lead in establishing and implementing the international guarantees and controls which will prevent its future use." Unfortunately, despite these forcible exhortations the political environment of the world remained such that it was necessary for the United States to continue study of the problems concerned with potential use of nuclear weapons.

The introduction of radiation studies into the activities of NMRI resulted from the organization by the Bureau of Medicine and Surgery of the U.S. Navy of a unit determined to solve the medical problems created by the use of the new weapon. On 8 September 1945 Comdr. Shields Warren, MC, USNR, was ordered to NMRI to implement the plans for the study of survivors in Japan. This medical group was integrated at Pearl Harbor with the Naval Technical Mission to Japan. From a study of the reports of the Naval Technical Mission to Japan it is clear that the United States received the first clear estimate of the effects of atomic bombs on cities. The pattern of injuries was shown to follow a definite chronological order based on the type received. The complications introduced by combined thermal, mechanical, and radiation injuries were clearly presented. The clinical course of uncomplicated whole body radiation injury produced by exposure to the initial bomb radiation was described. These initial classified reports clearly presented the medical effects of nuclear bombs. However, with the formation of a United States-Japanese Commission for the Study of Atomic Injuries under the Supreme Command the initial Navy studies were combined with this more comprehensive project. In addition to the purely biological effects of radiation, the Naval Technical Mission undertook a study in collaboration with Japanese physicists of the residual radioactivity. In this study survey instruments constructed at NMRI were utilized by Lt. Comdr. N. Pace, H(V)S, USNR, and Lt. R. E. Smith, H(V)S, USNR, in this first field study. The half-life of residual activity was successively determined and a measure of the intensity of the fallout in Nishiyama Reservoir area at Hiroshima was made and shown to be insignificant. This first study of fallout intensities was later to become useful in 1958–62 studies by Atomic Bomb Casualty Commission in Japan.

As a result of the strategic bombing survey, the Joint Commission on the Study of the Effects of Atomic Bombs and the Naval Technical Mission to Japan became clear that further information was needed in respect to the effects of nuclear bombs and in particular, to the quantitative dose effect relationships between nuclear radiations and the biological response of living objects and in particular human beings. For this purpose a joint planning committee under the Joint Chiefs of Staff headed by Maj. Gen. C. E. LeMay, USAF, was developed. The predominance of Navy interests, in tests of the kinds contemplated, resulted in the appointment of Vice Adm. W. H. P. Blandy, USN, as the Commander of Joint Task Force One.

The need for the tests grew from the fact that information on the effect of atomic bombs upon naval vessels and shipping in general was almost wholly lacking. Such a study was required by the defensive interest of the United States. The Joint Chiefs of Staff planners decided that three tests should be carried out including an air detonation with second priority to a surface or underwater detonation, and

third priority to detonation at a depth many thousands of feet beneath the surface. The specific objective of the tests would be to ascertain the strategic and tactical significance of the atomic bomb as affecting the future composition and employment of armed forces and determine what changes would be required in naval design and construction. Since ionizing radiation had become clearly a new type of casualty producing agent, biological studies were to be an integral part. The Naval Medical Research Section of Joint Task Force One was organized under Rear Adm. T. A. Solberg, USN, Director of Ship Material, by, Capt. R. H. Draeger, MC, USN, of the Naval Medical Research Institute, who was appointed as Commanding Officer and Capt. Shields Warren, MC, USNR, who was appointed as Executive Officer of the Naval Medical Research Unit. As a result of his known distinction in radiation pathology and participation in these tests, Warren later became the first Director of Division of Biology and Medicine of the U.S. Atomic Energy Commission. The U.S.S. *Burleson,* APA 67, was designated as animal and laboratory ship and ordered to the U.S. Naval Shipyard, San Francisco, for remodeling into a sophisticated laboratory and animal quarters afloat, after designs by Capt. R. H. Draeger, MC, USN and Lt. S. Seal, MC, USNR.

The biomedical program was designed to test the effectiveness of radiation against living animals and to study thermal burns and blast effects. Aboard the ship were 200 swine, 204 goats, 60 guinea pigs, 5,000 rats, and 200 mice to be used in the studies. Major accomplishments of the radiobiological program were: (*a*) The determination of the inability to predict satisfactorily the dose of radiation within ships thus demonstrating the absolute necessity for development of satisfactory dosimetric techniques. (*b*) That radiation could be a lethal agent under conditions where ships are not destroyed and individuals beneath decks are protected from thermal and traumatic injuries. (*c*) Several new dosimeter devices and methods were tested and shown to be of limited effectiveness. (*d*) Following test BAKER, the underwater shot, the highly lethal nature of radiation from the base surge and rainout over a wide area was demonstrated in ships, with animals beneath decks, in which the ships suffered minor physical damage but in which the animals died within 3 to 4 days following the exposure from pure radiation injury. (*e*) The flora and fauna which grow upon the bottoms of naval vessels were shown to concentrate significant radioactivity from the contaminated sea water. (*f*) The large animal studies at Operation CROSSROADS clearly demonstrated the feasibility of dividing radiation injury into various categories depending upon the rapidity of the development of leukopenia and the tempo with which the signs and symptoms appear. (*g*) Furthermore, it appeared, although there were inadequate control studies, that antibiotica and fresh blood transfusions were beneficial. (*h*) The predominance of gastrointestinal symptomatology and injury in the very high dose groups was suggested. (*i*) In the lethal dose range where there was a spontaneous possibility of survival, the predominance of overwhelming infection was conclusive. The pathology and lethal dose studies clearly showed that an underwater atomic bomb explosion produces much more lethal ionizing radiation over a larger area due to the base surge and the radioactivity that comes down in the rainout. (*j*) Pathology studies gave confirmation of the clinical and hematological studies during life. The histopathological studies of Lt. Comdr. J. L. Tullis, MC, USN, on the Bikini animals pointed out for the first time an important exception to the law of Bergonie and Tribondeau, namely, that the stem cells of the lymphoid organs, the bone marrow, the testes, and the ovaries are more resistant than the more mature cells. This important basic observation in radiation biology was made as a result of applied studies in the field.

Partial or complete failure to attain certain objectives clearly define certain problem areas: (*a*) The quantitative relationship between the dose of radiation and ultimate mortality in different species of animals was not determined because of failure of radiation dosimetry and/or the difficulty of computing the dose beneath such a complicated shielding system as exists in naval vessels thus demonstrating a great need for development of refined dosimetry of the initial bomb radiation and residual radiation fields. (*b*) The complicated shielding configurations from ships' compartments, bulkheads, and machinery which "shadow shielded" animals demonstrated the necessity for performing inhomogenous irradiation of animals. (*c*) The necessity for dose rate and lethal dose studies combined with histopathologic and hematologic studies was clearly demonstrated after the BAKER contaminating test. (*d*) The presence of combined thermal, mechanical, and radiation injuries was shown clearly thus indicating a new field for study in the laboratory.

The serious health problems of surface ship radioactive contamination, and entrance of fission products into the life cycle of marine flora and fauna growing on ships' bottoms were problems that could not be handled at NMRI and necessitated a new laboratory embracing a cross-disciplinary attack of physics, chem-

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istry, engineering, and biomedicine. Thus the U.S. Naval Radiological Defense Laboratory was born.

The positive accomplishments and the failures to attain some objectives clearly aided in the design of continuing studies at NMRI in preparation for later field testing if further nuclear bombs were to be exploded. Upon the return of the Naval Medical Research team to NMRI, the Atomic Medicine Division under Capt. R. H. Draeger, MC, USN and Comdr. R. H. Lee, MSC, USN, was formed in order to plan for further field tests. Intramurally systematic pathological and hematological studies on swine, goats, dogs, and small mammals in order to dissect systematically the pattern of radiation injury as a function of dose, percent mortality, varying depth dose patterns, and varying dose rates were commenced. In conjunction with these investigations, comprehensive depth dose studies were instituted by the Radiation Technology Division under Comdr. W. H. Chambers, Jr. MSC, USN.

The internal collaborative studies between divisions at NMRI rapidly began to bear fruit. As a result of collaboration between the Pathology and Radiation Technology Divisions, the vital role of differing depth dose patterns upon the mortality of radiation was demonstrated clearly. For the same amount of radiation delivered in air a more homogenous distribution within tissue is significantly more lethal. These studies clearly pointed out the necessity of eventually determining the depth dose pattern of the initial neutron and gamma radiation from nuclear bombs and the depth dose pattern from radiation that might develop in any military situation in order to assess the military hazard. Thus one was confronted with two primary and equally important problems. First, it was necessary to work out techniques for delivering reproducible depth dose patterns in animals from whole body radiation in order to study hematological and pathological effects and to evaluate potential therapeutic or prophylactic agents. Second, it was necessary to work out the techniques for practical determination of depth dose patterns for the laboratory which could be used in the field while testing future atomic bombs.

For the dosimetry, the Radiation Technology Division developed small Sievert type ionization dosimeters that could be placed at successive depths in tissue equivalent phantoms for measuring the tissue dose. In addition, a sophisticated array of film and phosphor dosimeters were also developed for use in parallel with the small ionization chambers. With these techniques, precise measurements of the distribution of the dose within tissue equivalent phantoms were made for the 1-Mev X-ray machine at the Naval Gun Factory, the 2-Mev X-ray machine at the Naval Ordnance Plant, and the 250-kvp therapy unit at NMRI. In addition they were utilized to measure the air dose and scatter from the exposure equipment under different conditions of scattering material. The role of scattered radiation and the careful design of exposure equipment to assure reproducibility and to include scattered radiation in the biologic effects were successfully exploited by F. Ellinger. It was shown early that the most practical means of having a reproducible depth dose pattern in animals of varying sizes was to deliver half the dose to each side of the animal. This technique was thoroughly evaluated from a biological standpoint and shown to be acceptable for controlled studies of radiation mortality and therapy of radiation injury and has now become a standard technique in radiation biology laboratories.

The radiation from a nuclear bomb is directional and it was necessary to try to simulate bomb radiation. In order to accomplish this it was first necessary to await field tests in order to determine the depth dose pattern of the initial radiation from a nuclear bomb and also to see if depth dose pattern varied with distance from the explosion point. An opportunity to initiate these studies would not appear until 1951 during Operation GREENHOUSE.

Between 1946 and 1949 a broad biological program on the hematological and pathological effects of radiation and the modification of radiation injury by chemical means was launched jointly by the Hematology Division (E. P. Cronkite) and Pathology Division (J. L. Tullis). For chemical prophylaxis against radiation a program was developed for study of sulfhydryl compounds which was based upon the works of E. S. G. Barron of the University of Chicago (consultant to NMRI) who had demonstrated that the addition of glutathione (GSH) to enzymes would afford considerable protection against the inactivation of these enzymes by radiation in vitro. However, in order to evaluate the effect of glutathione (or other substances) it was first necessary to establish precise methods of bioassay of radiation effects. This involved not only precise techniques for the irradiation of the animals but also a very careful randomization and statistical selection to avoid introducing bias into the experiments, and careful maintenance to prevent introduction of infection into the irradiated colony after exposure. Utilizing the radial beam of the 2-Mev X-ray machine at the White Oaks Naval Ordnance Laboratory it was possible to expose animals at dose rates

of 32 r per minute and simultaneously expose up to 512 animals so that an entire LD_{50} curve could be determined simultaneously for the treated and control animals. It was demonstrated that glutathione when administered prior to irradiation would significantly protect, almost doubling the LD_{50} dose of irradiation. However, the amounts of glutathione that produced this degree of protection verged on being lethal themselves. Independently and shortly before demonstration of protection by glutathione at NMRI, the Argonne National Laboratory demonstrated protection by intravenous cysteine. These were the first two clear-cut demonstrations of the capability of protecting against radiation by the administration of chemical substances prior to irradiation. Systematic studies were performed on the nature of the sulfhydryl protection. It was demonstrated that the protection was obtained only while a significantly increased concentration of sulfhydryl compounds existed within radiosensitive tissues essential to life. It was further shown that only those tissues that concentrate the sulfhydryl compounds are protected. For example, the testicle does not increase the concentration of glutathione following its administration and is not protected. The preceding was based upon extensive studies of tissue distribution and plasma clearance in diverse species and then selected studies upon the rate of development of atrophy of tissues following irradiation.

When a clinically interesting compound was reported in the literature as having a protective effect against radiation, it was checked with the techniques available at NMRI for assay in irradiated mice. It was successively demonstrated that none of the rutin-flavonoid group of compounds were of benefit as claimed by others. It was further demonstrated that vitamin B_{12}, folic acid pyridoxine, and other substances reputedly of benefit were in reality, when adequately tested in a statistically sound system, not protective.

Attempts were made to develop oral sulfhydryl compounds in conjunction with the Schwartz Chemical Co. but this was unsuccessful. The sulfhydryl protection which initially appeared promising and possibly useful in protecting military personnel against radiation was given up because of the high toxicity and the inability to maintain a prolonged protective level of sulfhydryl compounds in the tissues of irradiated animals. It is of interest that the entire sulfhydryl protection program was reopened by the U.S. Army Medical Department in an extensive program aimed at obtaining protective compounds. The pathological picture of sulfhydryl protection was performed in connection with G. Brecher at the National Institutes of Health (NIH). This study demonstrated the anatomical site and the cells from which hematological regeneration commences and generated a long and fruitful series of collaborative studies between Hematology Division, NMRI, and Pathology Division, National Institute of Arthritis and Metabolic Diseases, NIH.

Also in this period the Pathology Division systematically studied the pathology of radiation injury particularly of the gastrointestinal tract as a function of the dose of radiation. Later studies by Capt. R. B. Williams, MC, USN, 1955–60, on the quantitative effects of radiation on cell proliferation in the gastrointestinal tract have become classical basic studies on the effects of radiation on mitosis, regeneration, and DNA synthesis in the bowel.

A clinical analysis of the reports of the Joint Commission and of the Atomic Bomb Casualty Commission were undertaken and from these and studies on laboratory animals the now useful clinical subdivision of the radiation syndromes were developed and later were incorporated into the USAEC's test on "Effects of Nuclear Weapons." It was shown for practical purposes that one can divide radiation injury of man into three categories: (i) survival improbable, (ii) survival possible, and (iii) survival probable. Simple clinical observations determine in which category a patient probably belongs thus setting a basis for triage in the event of mass radiation casualties. It was further shown simultaneously at NMRI and elsewhere that radiation syndromes in animals vary with the dose of radiation received and the time after exposure. After very high doses of radiation of the order of many thousands of roentgen units either to the head or to the whole body, a typical syndrome develops characterized by signs and symptoms associated with the central nervous system and thus was called the CNS syndrome. This appears either during or shortly after exposure and is uniformly fatal. After doses of 800–2000 r, a symptomatology develops that is characterized by gastrointestinal symptoms which include a stable survival time in mice of 3 to 4 days and has in general a 100 percent mortality in all laboratory animals. In 1953 and 1954, it was shown by Conrad et al. that extensive administration of plasma and fluids to dogs with otherwise fatal gastrointestinal syndrome, would prolong life by preventing death from shock and thus allow sufficient time for spontaneous regeneration of the bowel from the histological standpoint. This was the first demonstration of successful therapy of the gastrointestinal injury. The pathologic studies on

bowel regeneration were another extension of the collaborative work commenced earlier with NIH.

After doses of radiation in the lethal dose range it was clearly shown that the gastrointestinal symptomatology is relatively fleeting and that the cause of death is related to the aplasia of the bone marrow, producing successively, increased susceptibility to infection that may have a fatal outcome within 1 to 2 weeks and later an increased susceptibility to spontaneous bleeding. Thus in the lethal dose range the first cause of death may be infection and later exsanguinating hemorrhage or fatal hemorrhage into a vital organ. This classification of the radiation syndrome produced by whole body irradiation is in general use today.

The Pathology Division evaluated the effect of radiation upon phagocytosis by the reticuloendothelial system utilizing the clearance rate of radioactive colloidal gold from the blood stream. At no time following total body X-radiation was the rate of removal of radioactive gold from the circulation found to be significantly impaired and it was concluded that total body ionizing radiation injury in the lethal range does not influence significantly the capability of the RES system to phagocytize foreign material within the blood stream. This was the initial study on the effect of radiation upon the capability of the reticuloendothelial system to phagocytize. Subsequently, numerous studies in many laboratories throughout the world have essentially confirmed this original work.

The Dental Division under Capt. James English, DC, USN, extensively studied the effects of ionizing radiation upon developing teeth in rats and in swine. In addition, studies upon the composition of saliva were also made. These studies have become classics in the field of the effects of radiation on oral tissues.

Among the oral manifestations of total body irradiation as seen in Nagasaki and Hiroshima patients was the presence of acute fulminating necrotizing gingivitis plus ulceration of the buccal mucosa. It was apparent that more basic information was required. Collaborative studies with the Hematology Division, NMRI, showed that in total body X-ray irradiated dogs, ulcerative gingivitis developed during the early stage of hemopoietic depression, reaching the fulminating stage as the animals became moribund.

The Bikini test trials presented an opportunity to observe the effects of high energy gamma irradiation. These studies showed that the ameloblast was especially susceptible to injury, having a pronounced effect on the developing tooth. Hemorrhage within the follicular sac was a common observation.

The effects of bilaterally applied X-rays to the head and neck of dogs, in doses ranging from 1000 to 1750 roentgens, yielded further valuable information. Salivary gland parenchyma showed evidence of severe injury, followed by bizarre changes in glandular cell architecture. Cell damage proved to be irreversible at higher dose levels. Dosimetry measurements indicated summation at the midline.

The metabolism of exteriorized salivary glands in the rat was affected by X-ray irradiation, these changes being especially noticeable in various enzyme systems.

Field studies at Frenchman's Flats revealed that many dental materials became dangerously radioactive after capture of thermal neutrons when released by nuclear explosions. Current studies are now in progress regarding the effect of thermal and fast neutrons on oral tissues and dental restorations.

A series of studies was performed on irradiated animals in order to determine whether radiation was different from any other type of stress. It was clearly shown that radiation did not differ significantly from other types of stresses, that adrenalectomy sensitizes animals to irradiation, and that there is a significant increase in excretion of 17-ketosteroids in the urine.

The Pathology Division (Brown, Hardenbergh, and Tullis) systematically studied the influence of irradiation upon the biochemical, cellular, and bacteriological content of thoracic duct lymph and blood in normal dogs and in dogs exposed to 500 r total body X-radiation. The white blood cell concentration of lmpyh dropped precipitously and attained minimum values within 4 hours after radiation and remained at this low level throughout the period of observation extending as long as 4 days after radiation. The cultures from blood and lymph remained sterile indicating that phagocytosis was not impaired since the animals did develop known infections.

In 1949 Operation GREENHOUSE was established as a joint Army-Navy-Air Force operation for the study of nuclear bomb effects. This turned out to be the operation in which the first thermonuclear bomb was exploded. Biomedical studies were a major part of the operation. Since the operation was to take place over a prolonged period of time and a laboratory ship had been found unsatisfactory at Operations CROSSROADS animal colonies and laboratories were built on Japtan Island at Eniwetok. These biomedical studies initiated a long and profitable cooperative program between NMRI, Los Alamos Scientific Laboratory (LASL), U.S. Naval Radiological Defense Laboratory (USNRDL), and the Division of Biology and Medicine of the U.S. Atomic Energy Commission (USAEC). The Operation GREENHOUSE bio-

medical program was a major undertaking in terms of personnel and money for each of the participating laboratories and involved the majority of the staff of the NMRI Atomic Medicine Division. The objectives of the radiobiological parts of the biomedical program were: (*a*) The determination of the LD_{50} of atomic bomb gamma radiation on large animals and on mice; (*b*) the determination of whether the rapid dose rate was more or less effective than the ordinary dose rates used in the laboratory; (*c*) the relative biological effectiveness of high energy gamma radiation; (*d*) the relative biological effectiveness of the fission neutrons from the bombs; (*e*) the correlation of the pathology of radiation injury with the clinical course; (*f*) the quantitative hematological correlation with ultimate mortality; (*g*) sophisticated fundamental studies when practical in the field; and (*h*) a long-term study of the surviving mice to determine the late effects and, in particular, dose effect relationship with development of all types of cancer. Because of a general policy of the Department of Defense the studies on carcinogenesis were not approved for NMRI. Accordingly, it was necessary to find a satisfactory laboratory to undertake these studies on the surviving mice. Fortunately, Jacob Furth of the Biology Division, Oak Ridge, agreed to undertake this initial study. The objectives were clearly evident to the biomedical groups at NMRI, USNRDL, and LASL. In order to attain these objectives numerous planning groups were formed and ultimately G. V. LeRoy was appointed as Director of the Biomedical Program directly responsible to the Task Force Commander. Primary responsibility for different aspects of preparation were assigned to different laboratories. The development of equipment for shielding against gamma radiation was primarily a Los Alamos project. Studies on the requirements for exposure conditions of swine and dogs in the tropics were performed primarily at USNRDL. Control studies on the mortality of radiation for dogs, swine, and mice were performed at NMRI. NMRI accepted the responsibility and through the Bureau of Medicine and Surgery organized BuMed Unit One which proceeded to Eniwetok Atoll and participated in the building of laboratories and animal facilities and the breeding of extensive colonies of LAF_1 mice, swine, and dogs for the experimental studies. The subsequent operation of the colony was a joint NMRI and USNRDL project. The mouse colony produced 16,000 healthy hybrid LAF_1 mice on time for use at the specific weapons tests. The large animal colony produced 291 dogs of the appropriate size and 300 swine for the tests on schedule. The total estimated cost of this formidable task on a tiny island in the mid-Pacific for the fiscal years 1950 and 1951 was $3,320,000. The later highly successful studies on the biological effects of radiation would have been impossible without the successful completion of NMRI's and USNRDL's mission in providing the required number of animals of desired age and size on specific dates.

The animal exposure equipment was designed and in part built at NMRI in order to protect animals against the thermal blast, and secondary missile effects for the study of radiation injury. This part of the program under Draeger's direction was highly successful. All equipment operated satisfactorily, attained the aims, and also protected the animals against the harsh environmental conditions of the tropical sun. Depth dose studies in tissue equivalent phantoms were carried out at NMRI under the direction of Comdr. W. H. Chambers, MC, USN. Its initial partial success formed the basis for further more refined and sophisticated studies during field tests in Nevada by Chambers and his radiation technology group. The results obtained at Operation GREENHOUSE were manifold of which perhaps the most significant attainment was the demonstration that competitive large laboratories such as NMRI, USNRDL, and LASL could work in harmonious and fruitful collaboration in the field, many thousands of miles away from base operations. The concrete scientific accomplishments were: (*a*) Establishment of the LD_{50} of atomic bomb gamma radiation for mice, dogs, and swine; (*b*) The high dose rate of atomic bomb's gamma radiation was shown not to be signficantly different in biologic effect than the ordinary dose rates used in the laboratory; (*c*) A first estimate of the relative biological effectiveness of the fast neutrons was obtained primarily by the Los Alamos scientific group and shown for acute effects upon spleen-thymus weight not to be in excess of 2; (*d*) It was clearly demonstrated that hemorrhage and infection were the major causes of death in the lethal dose range in swine and dogs; (*e*) The granulocyte count was shown to be a very useful sign to prognosticate the probability of survivors. Similarly, platelets were also found to have a clear prognostic value; (*f*) The role of the thrombocytopenia was clearly demonstrated as being of major importance in determination of the cause of radiation bleeding; (*g*) With the clear establishment of the major causes of death in the lethal dose range, studies on therapy of radiation injury were clearly pinpointed to evaluation of the role of platelet transfusions, leukocyte transfusions, and antibiotics.

Prior to concentration of efforts for Operation

GREENHOUSE by the Hematology Division, the problem of radiation hemorrhage was attacked. It had been published by others elsewhere and widely accepted that radiation hemorrhage was primarily due to "heparinemia" and secondarily due to thrombopenia. Whereas this concept was accepted at Operation CROSSROADS further studies at NMRI led to the belief that "heparinemia" rarely if ever developed in the irradiated dog. Since other NATO member nations were contemplating stockpiling of antiheparin drugs, a comprehensive attack on heparinemia was commenced. In this study an officer of the Royal Army Medical Corps participated, Maj. R. T. Lundie, in part along with G. V. LeRoy. The heparinemia concept was shown to be incorrect and antiheparin agents to be of no clinical value in dogs. Thus efforts on the study of the thrombopenia were intensified.

In 1949 it was recognized that a reliable method for platelet counting was needed before one could study reliably the relationship of platelets to bleeding. In conjunction with Brecher of NIH, a method for the enumeration of platelets involving dark phase contrast microscopy and a new anticoagulant was developed which has become a widespread platelet counting method throughout the world today.

While the bulk of the radiobiology staff of NMRI was at Eniwetok for Operation GREENHOUSE, a small part of the Hematology Division continued studies on the nature of radiation hemorrhage. It was clearly established that the tendency to bleed in irradiated animals was correlated with the thrombocytopenia and that all in vitro coagulation tests could be brought to normality by the addition of separated platelets.

Upon return of the Hematology Division to NMRI and upon completion of the Operation GREENHOUSE reports, the endeavors were aimed at establishing an effective therapy of radiation injury. It appeared logical that platelet transfusion, leukocyte transfusions, and antibiotics would be effective against radiation injury. The first aim was the development of methods for platelet transfusions. First, and again in conjunction with Brecher of NIH, a method for the satisfactory separation of platelets was accomplished. Parenthetically, this method with minor changes is still in use in clinical medicine for platelet transfusions today. Second, the effectiveness of transfusions of fresh platelets into irradiated animals at levels of radiation known to produce 100 percent bleeding was evaluated. It was conclusively demonstrated that platelet transfusions would prevent the development of bleeding in animals provided a platelet count be maintained above about 50,000 per cubic millimeter. Furthermore it was shown that platelet transfusions could stop bleeding that had already commenced. This was the first clear-cut demonstration of the unequivocal role of the platelet in the pathogenesis and the prevention of radiation bleeding.

During this same time period collaborative studies with K. M. Brinkhous, University of North Carolina, demonstrated that antihemophilic factor (AHF) was not involved in radiation hemorrhage thus showing in a basic study that AHF is not produced by lymphocytic tissues since the studies were performed while the lymphocytic tissue was aplastic. Studies in collaboration with L. M. Tocantins, Jefferson Medical College, on plasma antithromboplastin were commenced and shown to be increased.

Next, methods for the separation of granulocytes from fresh canine blood were also developed in collaboration with Brecher, NIH. It was shown that the transfusion of freshly separated granulocytes into irradiated dogs would reverse the histologic picture. The transfused granulocytes migrated to sites of infection and successfully prevented the widespread dissemination of bacteria. However, the animals would then die from extensive hemorrhage. The studies on platelet and granulocyte transfusions clearly demonstrated the essential role of these circulating cellular bodies in the pathogenesis of radiation hemorrhage and infection but unfortunately were not successful therapeutically because the levels of irradiation were inadequate to completely suppress the immune responses and after 2 to 3 weeks antiplatelet and antigranulocyte substances were produced making it impossible to continue to maintain satisfactory levels of platelets or granulocytes; thus the animals died. Preliminary studies on the combined use of fresh blood and antibiotics suggested that these would be of value in increasing the survival rate of otherwise fatally irradiated animals. This has subsequently been thoroughly demonstrated in many other laboratories.

The studies performed elsewhere had shown that the original Jacobsen (University of Chicago) concept of humoral protection against irradiation by splenic suspensions was not correct and that bone marrow or spleen cell suspensions protected by transplantation of a stem cell that repopulated the depleted bone marrow. At this time an extension of the collaborative work with NIH involved a study of the influence of parabiosis upon survival of irradiated rats. Parabiosis was initiated before irradiation and only one parabiont was given a fatal dose while the other was lead shielded. The protection was dramatic and

significantly proved that the protective cell or "humor" moved through the blood. Subsequent studies elsewhere conclusively proved the protection to arise from stem cells that can be concentrated from normal blood. The surviving parabiotic protected rats were studied. These animals had been protected from an otherwise fatal dose. A striking induction of cancer other than leukemia was observed.

In order to study further the biological effects of gamma radiation under laboratory-controlled conditions, the Atomic Medical Division under Draeger, designed and completed in 1952 a 1000 curie cobalt-60 irradiator. This source was unique in that an animal as large as a swine could be exposed to uniform gamma rays from a 4π solid angle. Installed in a specially-built structure, the cobalt was transferred between two shielded rooms to form a cylindrical pattern around the exposure chamber. Two years later, the cobalt-60 was replaced to increase the source to 2500 curies. Many biological specimens, animals, materials, and clinical patients have been exposed in this gamma ray generator.

In continuing field tests that were being planned for Nevada there were evidently two things that needed to be studied further. First, the problems of dosimetry initiated at Operation GREENHOUSE by the NMRI group under Chambers needed to be extended and confirmed. In Operation BUSTER the Radiation Technology Division clearly established the depth dose curve of atomic bomb gamma radiation in tissue equivalent phantoms. In laboratory studies it was clearly shown that this depth dose pattern could be simulated by the 10-Mev betatron at the Naval Ordnance Laboratory (NOL). At Operation UPSHOT-KNOTHOLE in 1953 the atomic bomb gamma radiation and depth dose from fallout was studied by NMRI's Radiation Technology Division. This now classic picture of the flat depth dose curve in a fallout field was established and is commonplace in texts today. This is of particular significance because at the time of Operation UPSHOT-KNOTHOLE there was no clear appreciation that fallout was to become later a significant hazard to human beings since the accident of Operation CASTLE in 1954 had not yet happened. It is of interest to record the conclusions of the Radiation Technology Group in 1953. "The depth dose curve of the high energy component in the case of a fallout area 3.5 miles from ground zero shows a more uniform distribution of dose throughout the phantom than does the depth dose curve obtained from the initial radiation. Therefore, the effects of a given dose of radiation from a residual field could be more serious than those from the initial radiation because of (*a*) the relative uniformity of the field of radiation which produces a more uniform dose throughout a man's body, and (*b*) the additional presence of the readily absorbed radiation." In addition to the establishment of the flat depth dose curve from a fallout field beta/gamma ratios were also presented. The preceding is a distinct example of the forward thinking in planning and making physical measurements of biological importance before the establishment of a real biological problem by experience or accident.

In Operation TUMBLER-SNAPPER there was again a fruitful collaboration between NMRI and USNRDL aimed towards establishing firmly the relative biological effectiveness of the neutron component of fission bombs. This was successfully accomplished showing the RBE to be approximately 1.6. In addition, for the first time, the peculiar neutron induced 3- to 4-day death of mice, was established. In addition, during Operation TUMBLER-SNAPPER biological studies on mice demonstrated the presence of an as yet unknown high flux of neutrons that could be a casualty producing agent to troops in the field. This was shown prior to physical measurements and computations in conjunction with the testing of this experimental tactical weapon. This emphasized the significant contribution of biological studies to the understanding of weapons effects. The mouse is a superb integrating radiation dosimeter under certain conditions.

In early 1952 the USAEC was planning a series of experimental and test explosions in which a major effort would be devoted to making studies pertinent to civil defense. The USAEC requested the Department of the Navy to permit Comdr. E. P. Cronkite, MC, USN, of NMRI to be the program director for the biological programs and to organize a collaborative study between NMRI, USNRDL, Oak Ridge National Laboratory, LASL, Lovelace Foundation, and various universities that desired to participate in basic scientific radiobiologic studies and in the evaluation of the AEC prototype shelters in respect to protection against blast, thermal, and ionizing radiation. A comprehensive program of basic and applied nature was formulated and satisfactorily executed and reported. The radiobiologic studies performed jointly by NMRI and USNRDL showed that the prototype shelters were exceedingly good shields against gamma rays and neutrons. The other objectives of field testing listed earlier with the exception of the depth dose pattern of fast fission neutrons were repeated with the same re-

sults. This field atomic bomb study clearly proved the feasibility of civilian laboratories and university scientists working under a military direction in relative harmony and with definite accomplishments.

Before and during Operation UPSHOT-KNOTHOLE the Oak Ridge group had developed a method of measuring neutron flux and energy by fission foil detectors. In 1955, utilizing the NMRI gamma detectors and the Oak Ridge fission foil detectors, an NMRI group under G. Imirie and the Brookhaven National Laboratory group under V. P. Bond studied the comparative neutron and gamma depth dose patterns. These studies were highly successful and proved that gamma and neutron radiation cannot be added rad for rad, corrected for RBE in thin foils in air to a meaningful dose because the neutron depth dose curve falls off much more rapidly than gamma depth dose curve, thus indicating that per rad fast neutrons probably are not as lethal as penetrating gamma rays.

Operation CASTLE (1954) was designed as a full scale test of thermonuclear devices. There was no planned biological program by NMRI. On 1 March 1954, following the detonation of a 15 megaton experimental device at ground level an unexpected change in the wind pattern resulted in the deposition of large amounts of fallout upon atolls inhabited by the Marshallese, American servicemen operating a weather station, upon the task force, and upon a Japanese fishing vessel, the Lucky Dragon.

The task force was able to wash down satisfactorily and personnel aboard received no significant radiation injury. However the Marshallese and American servicemen were exposed to potentially dangerous amounts of radiation from the fallout before they were evacuated by plane and ship.[1]

The task force commander requested assistance from the USAEC for care and study of the irradiated individuals. The Director of the Division of Biology and Medicine of the USAEC, J. Bugher, turned to the NMRI and requested through the Surgeon General that Cronkite, be appointed as officer in charge and that he organize a medical team from the experienced personnel of the U.S. Navy. This study became again a joint NMRI–USNRDL project with Bond as assistant project officer representing USNRDL. Within 48 hours a team was assembled consisting of experienced M.D.'s, Ph. D.'s, radiation physicists, and technicians. The equipment was crated and airlifted to Kwajalein Naval Air Station to which the individuals had been evacuated. The clinical and hematological studies performed upon these individuals clearly established the symptomatology in human beings after an exposure of radiation approximately at the level of 175 rad. The sequence of events in the development of beta skin burns was documented and is now a classic study. The development of granulocytopenia, lymphopenia, and thrombocytopenia was clearly established and shown to be significantly different from that in experimental animals after comparable doses of radiation. The conservative management long recommended by the NMRI group was clearly shown to be the treatment of choice. The cardinal principles of management of radiation injury were established as meticulous history and clinical examination with attention to preexisting chronic infections, laboratory studies to estimate degree of bone marrow suppression and to avoid all therapy until something is clinically indicated. The exposed individuals experienced a severe epidemic of upper respiratory infection which also involved the staff and nonirradiated individuals thus demonstrating that prophylactic treatment of irradiated human beings by antibiotics is not necessary.

The conservative clinical management of the above individuals, in face of pressure to institute transfusions and antibiotic prophylaxis, as the granulocyte and platelet counts continued to fall, resulted in the now generally accepted policy of observe and wait for clinical indications for treatment that has subsequently been followed in most later radiation accidents.

In addition to the above clinical studies an opportunity for the study of internal contamination existed. The exposed people had lived, breathed, and eaten in a highly contaminated environment for over 48 hours. The degree of internal contamination of the people and animals was measured primarily by the USNRDL group. The studies pointed out, under the conditions that existed (breathing, ingesting, and living in a contaminated area) that the magnitude of exposure from external radiation greatly outweighed the magnitude of the internal contamination. In fact the former might well reach lethal levels whereas the latter by itself would produce little if any injury.

The body burdens of the various radionuclides were established and the studies were begun on biological turnover rates. The studies were continued by Brookhaven National Laboratories.

In 1955 and 1956 there were continuing field

[1] The following exposure groups were to be followed:
a. 28 American servicemen about 60 rads.
b. 64 Marshallese about 175 rads.
c. 18 Marshallese about 70 rads.
d. 157 Marshallese about 20 rads.

studies in Nevada in which the Radiation Technology and Pathology Divisions participated to complete the details on various of the objectives laid down following Operations CROSSROADS and GREENHOUSE.

Upon the establishment of the International Moratorium for testing of nuclear weapons experimental field work obviously terminated. The remaining problems of military importance in respect to radiobiology and radiation dosimetry clearly needed sophisticated experimental tools not available at NMRI. For this purpose radiation facilities (cyclotron and other devices) were approved for installation at USNRDL and a new laboratory was conceived, the Armed Forces Radiobiology Research Institute (AFRRI). The new laboratory would have not only a "flash" reactor but also high intensity gamma generators which could mimic the neutron gamma spectra of different nuclear weapons and thus evaluate their effectiveness as radiation antipersonnel weapons.

The serious scientific study of radiation effects upon man and mammals for the naval service commenced at NMRI in 1945. The radiation biology program consisted of basic studies on radiation, effects on mammals, radiation dosimetry, and the management of radiation injury. The laboratory program was always dovetailed with field studies at the Nevada and Pacific Proving Grounds aimed at the solution of military problems. The coherent laboratory and field research program attained its fullest appreciation just when the Navy, through NMRI, was requested to undertake the scientific direction of biomedical programs of Operation UPSHOT-KNOTHOLE for the Civil Effects Groups of the USAEC and second when the Navy again through NMRI was requested to take the responsibility for the care and study of human beings accidentally exposed to fallout radiation at the Pacific Proving Grounds. With the establishment of AFRRI the radiobiological program of NMRI was phased out logically and responsibility for its continuation assumed by AFRRI and USNRDL.

IV. AVIATION MEDICINE

H. G. Wagner

During World War II, aviation medicine was one of the first identified work areas at NMRI. There were a myriad of aeromedical problems faced by the operating forces. This brief review will attempt to discuss some of the more pertinent investigations. Due to the number of studies involved, it is necessary to be selective. More than 100 reports of completed research have been published on such diverse subjects as the development and evaluation of personal-survival equipment, mechanical-safety equipment, vision and retinal burns, training and performance of pilot under stress situations, hypoxia, oxygen equipment, training in techniques of utilization of oxygen equipment, carbon monoxide problems, high altitude and decompression problems affecting aviators, and noise and vibration. Only a few sample references will be given.

One of the principal work areas was the development and evaluation of equipment which would enable aviation personnel to have the maximum chance of survival should they be forced down at sea during their flight mission. Ballon-type locators and markers for use by personnel were evaluated as were various types of protective clothing and survival packets for use during the ordeal at sea. Armored jackets for use by aviation personnel were also evaluated as to feasibility and use.

Various configurations of flight goggles were evaluated in an attempt to eliminate fogging, improve vision, and increase visibility under different atmospheric conditions. Investigations were made in the use of oxygen equipment, particularly under conditions of stress such as in evacuation of submerged aircraft. The number of ditchings and forced landings in which the aircraft either quickly submerged or flipped on its back, required that investigations be made in this area and recommendations made as to the type of system and pilot technique to be used in such situations. Many of these techniques are still currently in use. As flight missions exceeded 12,000 feet altitude the importance of adequate oxygen supply to crewmen became critical. Face masks were evaluated and developed to assure the pilot of adequate freedom of movement, without interfering with his goggles (hence vision) and with a minimum of leakage. Not only Navy issue, but masks and suspensions in use by sister services and Allied forces were tested and modified. Techniques for cleaning and sterilizing these masks were developed and forwarded to the operating forces. Simulated bailout jumps and practice descents from altitudes of up to 35,000 feet using modified breathing procedures were carried out in the low pressure chamber. This was done to evaluate the feasibility of evacuating aircraft without the use of special bailout bottles. This study was not confined to the laboratory, but was also made under actual flight conditions. Diluter demand oxygen regulators were evaluated operationally in cooperation with the Tactical Test Section at Naval Air Station (NAS), Patuxent River. Comparisons between this system and other systems for transport-type aircraft were field tested on overseas flights from Patuxent River to Port Lyautey, North Africa. A constant flow-reservoir (rebreather bag) was evaluated both under laboratory and actual flight conditions as a means of determining the minimal oxygen flow delivered to the mask which would be required to produce adequate oxygenation.

Also of prime importance and consideration was the problem of carbon monoxide (CO). It was recognized that cracked exhausts or leaks in the firewall could permit accumulation of this toxic gas in the cockpit from the aircraft's engine exhaust. The rate of

blood absorption of various concentrations of CO and its effects on performance of personnel at altitudes of 10,000 feet and higher were of vital interest. Results of these findings were forwarded to the fleet for incorporation into safety directives. Not only were aviation personnel exposed to the hazards of CO while flying, but also when aboard carriers during warmup and launch of aircraft. To determine the concentration in these areas, a series of studies was made as to maximum time of warmup allowable for the type of aircraft and wind direction. Through the use of special devices and simulators, including the low pressure chamber and Link trainer, psychological and physiological reactions of subjects were evaluated under various degrees of stress. These studies included the effects of carbon monoxide on critical flicker fusion frequency, changes in body sway as measured by an ataxigraph, and also the effects of inspiration of low oxygen-high nitrogen mixtures. Of considerable interest was the effect of drugs and alcohol on the sensory-motor system. A unique study of their effects was made with the use of the Link trainer (532).

In an effort to improve conditions in simulated instrument flight instruction and at the same time improve safety while flying, several "blind-flying kits" were evaluated. An incompatibility became apparent in the blue-orange system of simulating instrument flight. With the blue goggles on, the pilot found it extremely difficult to read and interpret some instruments such as radarscopes, etc., which depended on long wavelength persisting phosphors. Initially, several devices including a duckbill visor baseball cap, venetian blinds, and near focus lenses were tested. Each of the devices tested presented a number of deficiencies. As a result, a new system of panels and a cap with movable visor was developed (41), which did meet the established criteria. The high fatality and serious injury rate in high-speed aircraft emphasized the need for more adequate personnel protection during the extreme decelerative forces which occur in crashes and arrested landings of the type experienced aboard carriers. The characteristics of these forces imposed on the human body during deceleration were studied by means of instrumented manikins in operational aircraft actually crashed under closely simulated flight conditions. A dropped device was constructed to provide laboratory simulation of rapid decelerative forces on subjects wearing seat belts and shoulder harness. Strain gage instrumentation and high-speed motion picture techniques permitted the study of reactions of the human body to various forces. Recommendations were made relative to the design and incorporation of safety devices and cockpit configurations. Development test and evaluation of various types of safety belts and shoulder harnesses were made in order to improve the restraining gear for aircraft pilots (113, 114, 115, 116, 117, 118, 119, 120, 701).

Through anthropometric techniques, measurements of aircrew and aircrew spaces were made in various types of combat aircraft to determine the freedom of movement necessary for satisfactory performance. A somewhat similar study involved the determination of boundaries of maximum area for operation of manual controls by the pilot (915, 918, 1674).

In an attempt to further decrease the accident rate in naval aviation, the differential accident liability among naval flight personnel was investigated (1235). Previous studies in industry indicated that in any given group some individuals contribute disproportionately to the total number of accidents. Through the use of "peer ratings," pilots evaluated other members of their squadrons both as to safeness and skill. Also studied was the pilot's attitude toward various aspects of naval aviation, his subjective evaluation of flight safety procedures, and his galvanic skin response as he discussed statements concerning these same items of flight safety. On the basis of this study, the use of peer ratings as a means of establishing criteria of differential accident liability was recommended. Further, all variables studied were shown to be effective predictors of both the flight safety and skill criteria.

Shortly after World War II, further airborne investigations in aviation medicine were carried out under Project RAM (Research in Aviation Medicine). This project provided a flying laboratory in the form of a specially modified C–54 (R5D) and a flight crew. Although based at the NAS Anacostia, and receiving program direction from Office of Naval Research (ONR), it became an intimate part of the aviation program of NMRI until it was dissolved in 1961. Initially, it undertook investigations on human factors affecting operational performance. This was later modified to include transmission of physiological responses from air to ground by electronic methods. Rapid developments enabled personnel engaged in this area to design, develop, and evaluate equipment for telemetering physiological parameters. In 1954, the objectives set forth had been achieved to the extent that the first physiological data telemetered in this country from a pilot in an aircraft to ground station was accomplished by personnel from NMRI. Among the parameters telemetered were continuous records of the heart (EKG), brain (EEG), respira-

tion, and temperature. Project RAM and the Aviation Medicine Division of the Institute participated in each of the Strato-Lab balloon flights (stratospheric balloon flights carried out by ONR). These flights were inaugurated to study the atmospheric conditions at very high altitude as well as the phenomena beyond. Need for adequate biomedical safety measures was recognized at the outset and monitoring of the well-being of the Strato-Lab occupants was a constant feature of each flight. On one occasion, a flight surgeon noting an unusual condition in the telemetered oxygen system controls, ordered the balloon to descend. Fortunately, the crew in the gondola reached a level low enough to breathe without their oxygen masks, for their supply had diminished to approximately 2 minutes due to a faulty regulator. Refinements on later flights permitted telemetry of data from an open basket gondola to ground control and then transmission via telephone land lines to NMRI. Further advances permitted the transmission for the first time of pulse rate and heart beat via television from an airborne vehicle.

Research in the possibility of long-range transmission of physiological data was begun with an electrocardiogram recorded on magnetic tape successfully transmitted from Naples, Italy, to the Naval Radio Station, Cheltenham, Md., via low frequency radio and then to NMRI. The quality of transmission was such that diagnoses were consistently accurate when made on the basis of the transmitted electrocardiograms.

The following year, EKG's of patients at Tripler General Hospital, in Hawaii were transmitted by cable to San Francisco, then by telephone line to Montgomery, Ala., where a guest cardiologist at the Atomedics Symposium made initial diagnosis. EKG's were transmitted from the U.S.S. *Franklin D. Roosevelt* at anchor in Athens, Greece, via single-sideband radio and were then relayed via phone patch to the laboratory at NMRI. Further research and refinements in multiple-channel capability permitted physiological data from a crew member to be transmitted via single sideband radio on two flight legs from NAS, Anacostia to NAS, Key West, and from NAS, Key West to San Juan, Puerto Rico. Three EKG's, one through each axis of the heart, and a respiration pattern were transmitted. The following day the same parameters, on a different subject, were successfully transmitted from the Naval Communication Station, Balboa, Canal Zone.

On May 4, 1961, Strato-Lab V was launched from the deck of the U.S.S. *Antietam* in the Gulf of Mexico. The occupants of the open gondola were instrumented for telemetering a variety of physiological data including EEG's, EKG, rectal temperature, and respiration. These data were automatically recorded and transmitted directly to monitoring personnel both aboard the carrier and in tracking aircraft. In addition to the physiological data, environmental information was also transmitted. The value of telemetric monitoring was demonstrated when it was noted that the faceplate temperature was questionable on one of the crew and he was notified to correct it. Failure of the faceplate and implosion at the altitude would have meant almost instant death. The balloon, a 10,000,000-cubic foot aerostat, reached an altitude of approximately 113,500 feet thereby establishing a world's record for manned balloon flights. Other firsts include: the largest balloon ever used in manned flight, the first manned balloon ascent from the deck of a carrier, and the highest ascent by man in an open gondola. Unfortunately the flight was not without incident, as Lt. Comdr. V. A. Prather, MC, USN, member of the balloon crew and a flight surgeon attached to NMRI, lost his life by drowning during recovery operations.

Another contribution to the development of telemetry systems was the actuation of a warning device by a change in pulse rate. Limits on pulse rate would act as the actuator for sensory stimulating devices which would warn of impending cardiac stress. A single EKG utilizing subminiature components was designed, developed, and successfully utilized as was a six-parameter field pack bioinstrumentation system for use in field operations.

In 1958, instrumentation of a monkey for space flight was carried out in cooperation with the Naval School of Aviation Medicine. The physiological data transmitted from the nose cone were received and recorded in an R5D flying laboratory. The nose cone of the capsule was not recovered; however, data recorded were available for scientific evaluation.

The advent of carrier-based aircraft powered by jet engines particularly those with afterburners gave rise to the question of potential hearing loss hazards to flight deck personnel. Since excessive noise is irritating, fatiguing, deafening, and in extreme cases, may produce outright injury, a noise level survey was carried out aboard the U.S.S. *Coral Sea* during routine refresher maneuvers (644). Observations were made of noise levels at a number of flight deck positions and various ship spaces. A result of this survey included recommendations relative to personnel protection, modification of operating procedures, communication

equipment and procedures, and serious consideration of this problem in future ship design.

Contaminants in the earth's atmosphere have, in the past, interfered with attempts to correlate the theories of light scattering with measurements of sky brightness. Further, little data on changes in brightness in the lower atmosphere incident to changes in altitude had been available. Equipment was developed, built, and installed in the RAM aircraft that would record brightness as fast as the aircraft could be flown through prescribed patterns at designated altitudes. Information was obtained on the measured brightness of the zenith, the nadir, and of points separated by 15 degrees of arc. The initial study reported data on clear days only. Subsequent reports demonstrated the effects of cloudy conditions on atmospheric brightness and indicated its extent and nature below cloud formations and the similarity between clear weather sky brightness and sky brightness above cloud overcasts (37, 38).

Efforts to increase safety of aircraft landings, particularly in conditions of low visibility, have centered largely in the development and utilization of electronic devices and high intensity approach lights. These devices have been very effective in reducing the hazards of such low visibility approaches. However, the final transition and landing must be made visually and minimums are established below which the pilot may not go.

One of the hazards facing air crewmen in an atomic war is retinal burn. Useful vision can be destroyed at distances far beyond the limits where thermal radiation or blast cause damage. This is because the eye images the explosion on the retina thus concentrating the energy in such a way as to coagulate tissues. An evaluation of the hazard of injury and possible protective measures would be of some assistance to an important Navy problem. The Institute has been focusing its research on the histopathology and functional disorder that will accompany this type of injury.

A new device of great potential value to the military is the operations Laser. This also will cause a retinal burn if directed against the eye. The phenomena are different in some respects from thermal injuries resulting from nuclear weapon explosions. The understanding of the hazards of Lasers is an important biomedical concern. The research on retinal burns is being conducted with particular attention to Laser-type injuries.

V. BIOPHYSICS

D. E. Goldman

Problems involving mechanical, acoustical, electromagnetic, and other physical forces in biology and medicine are of great importance to the Navy. However, little was done in this area at the Naval Medical Research Institute during World War II, partly because of the competition of other needs and partly because these problems were in an embryonic state. One study was carried out on blast injury to experimental animals (326) and one on the effects of impact resulting from underwater explosions (403). Toward the end of the war the advent of jet aircraft, together with other machinery of great power capacity, aroused concern as to the effects of mechanical forces on personnel, particularly the pilots and ground crews of high-powered planes. This led to studies on vibration, noise, ultrasound, and impact. The work on impact is covered in chapter IV.

In view of the scanty knowledge on the biological effects of mechanical forces, it has been necessary to carry out considerable basic research. A joint program was established with the Naval Research Laboratory (NRL) which led to the construction of a special vibration machine and of equipment for the study of ultrasound. In the meantime an analysis was undertaken of the subjective responses of personnel to low frequency mechanical vibration (640) and this led to the establishment of a preliminary basis for specifications and safety limits. A study was also carried out on experimental animals confirming previous German observations that the application of mechanical vibration to muscles and tendons interfered with stretch reflexes. The mechanism of the effect was shown to involve the reflex pathways (641).

With the installation of the vibration equipment a long-term study was begun on the physiological and pathological effects of mechanical vibration using anesthetized cats as experimental subjects. It quickly became clear that when animals were exposed to intense low frequency vibration the method of support of the animal was of primary importance and that it was quite possible for secondary injuries due to blows against the supports to exceed the injuries from the vibration itself. For this reason a technique was evolved of immersing the animals completely in water. This permitted observations on the effect of vibration itself to be studied without interference from secondary injuries. It was then found that damage from mechanical vibration resulted primarily from the vibration of the heart inside the chest cavity. Atelectasis and hemorrhage were produced in those areas of the lungs struck by the heart and some disturbances in heart function were found as indicated by electrocardiographic changes and occasionally by changes in the appearance of the cardiac muscle tissue itself. Respiratory air exchange was markedly decreased during exposure to vibration but overall cardiovascular function appeared to be fairly well maintained even in animals which died as the result of exposure. While an animal is not necessarily a good simulant for a man, the same qualitative behavior and general effects of mechanical vibration would be expected in humans (1234).

In the area of ultrasound a field study was carried out at the Philadelphia Naval Base on the exposure of humans to the noise and ultrasound from jet aircraft engine exhausts. This indicated that at the power levels then available the hazards of ultrasound were insignificant, although the intense noise clearly required the use of ear protective devices. At the same time laboratory work was undertaken on the acoustic properties of certain cells and tissues and on the effects of ultrasound on cell structure. Cells and cell suspen-

sions were exposed to standing wave systems at high frequencies. Injury occurred at equal (half-wave) intervals along the direction of the sound wave. Not only was there damage due to the acoustically generated cavitation but, if steps were taken to avoid the cavitation, there was also damage due to the wave motion of the fluid (643, 968). It was shown that certain cells, particularly spirogyra, were able to undergo partial recovery even though exposed continually to a high frequency sound field; or to put it another way, that exposure to a sound field reduced to some extent the sensitivity to further exposure, provided that the time sequences and intensities were appropriate (648).

Studies were also made on a method of detecting gall stones with the use of an ultrasound probe. This was shown to be possible but, in the current state of the art, not practical (1007, 1008). Later on, a collaborative project was undertaken with the Naval Dental School on the use of ultrasound for the preparation and drilling of teeth. Since studies on guinea pig incisors showed that the ultrasound produced damage to the odontoblasts, the use of ultrasound for tooth drilling was abandoned as being potentially hazardous (1315, 1316). However, ultrasound is now used in dentistry for superficial scaling of teeth.

About 1952 the problem of hazards from exposure to shortwave, high power electromagnetic radiation such as that used for radar became a problem due largely to the steady increase in the power capacity of generating equipment. A 2-year project was undertaken using several types of experimental animals with carefully controlled exposure conditions. The results (503) helped to establish the safety limit now in use of 10 milliwatts per square centimeter as a permissible exposure limit.

Parallel with these studies has been a program on the biophysics of nerve and muscle. Considerable work was done on the giant axon of the squid (1141, 1144). This included experiments on the behavior of the axon in vivo and computer analysis of some of the characteristics of the Hodgkin-Huxley formulation (270). In 1959 work was undertaken on the giant axon of the lobster which is obtainable in the Washington area throughout the year; squid is not. With the use of a double sucrose gap method, an artificial node was created in an unmyelinated fiber and it became possible to obtain fairly extensive data showing that the electrical behavior of the crustacean fiber was very much like that of the squid giant axon (888, 889). This same preparation was used to study electrical responses to mechanical stimulation. Rapid distortion of the fiber was shown to produce a depolarization which, if large enough, resulted in generation of an action potential (887). Slow distortion has no effect.

Work has also been carried out on a theory of nerve excitation in which the dipolar electrical properties of phospholipid play a large role.

In 1956, a detailed theoretical analysis was carried out on the effects of the extensive dendritic trees of nerve cells on electrophysiological data obtained in the central nervous system and spinal cord (1299, 1300).

Considerable work has also been done in the area of cell and tissue freezing and thawing (1100, 1101, 1102). A number of problems have been studied including the effects of freezing and thawing rates and of chemical additives on cell viability. The results have proven very useful for the understanding of mechanisms of freezing injury and of the practical problems of blood preservation (1104). Other applications of importance include the frostbite problem as encountered during military operations in Korea in 1952 (1093, 1098, 1103), the freeze-drying of biological museum specimens (1108, 1109), and the preservation of bovine spermatozoa (1107).

Studies were also made on the responses of muscle subjected to step changes in tension. A finite time is required for a stimulated muscle to reach its steady state condition. From the nature of the transients, a viewpoint was developed of the interaction of the thick and thin filaments of muscle in terms of a mechanochemical feedback system (1285, 1288). Later myofibrils were exposed to the direct action of electrolyte solutions showing that the presence of calcium was necessary for local contraction (1290). In another study it was shown that the exposure of muscle to very high pressures influenced the activation process of contraction in the same way as certain anions do. There is a possible correlation with the way in which the ions distort the water lattice in solution (1279).

VI. BIOENERGETICS

T. H. Benzinger

Human Gradient Calorimetry and Physiology of Temperature-Regulation

Work in the field of human calorimetry at NMRI was initiated in 1947 with the main objective of observing calorimetrically the heat loss or heat production which represents the effector system of human temperature-regulation. The method of gradient layer-calorimetry introduced by NMRI is based on the geometry of a blackbody, and on the design of a "gradient layer" (86, 96). The calorimeter consists of a cavity at constant temperature, in which the heat-producing object or subject is accommodated. The cavity is completely lined with a continuous layer of uniform low thickness and low thermal conductivity. The difference in temperature between the inner and outer surfaces of this layer is measured at approximately 6,000 sites of small, equal surface area. Heatflow from the human subject is intercepted by these measuring units. Through their electrical connection in series a single measurement of total heatflow is obtained, which is independent of the size or shape of the cavity and of the size, shape, location, or angular position of the heat-producing subject. Uneven distribution of surface temperature does not affect the measurement. With added gradient calorimeters for measurement of heat exchanged in circuits for the air that ventilates the calorimeter, or air exchanged in the respiration of the subject, cutaneous-evaporative and respiratory heat losses may be separately recorded. A rapidly responding and continuous measurement is thus obtained for the sum total and for the major components of human heat loss.

The gradient calorimeter has permitted for the first time rapidly responding, continuously recording, and partitioning measurements of human heat loss, including the sweating response (cutaneous evaporative heat loss) and vascular reactions (conductance derived from cutaneous heatflow and internal and surface-temperatures). Eardrum-thermometry, a technique developed at this laboratory, has permitted for the first time the continuous monitoring without anesthesia, of a cranial internal temperature representing patterns of temperature change in the central nervous system (97). This combination of new methods, with added conventional measurements of average skin temperature has resulted in finding quantitative relations between causes and effects, stimuli and responses in the thermoregulatory system of man which maintain body temperature close to a predetermined "setpoint" (92, 93, 94, 98, 99).

A terminal, internal warm-sensor of extraordinary sensitivity, with a sharply defined temperature-threshold maintains the internal cranial temperature—and thereby also body temperature in general—close to the individual setpoint of the "human thermostat" by two principal effector mechanisms:

(1) The sweating response (supported by centrally elicited vasodilatation) with a capacity of approximately 2 cal/sec. heat loss-increase (10 percent of a normal metabolic rate) for every increase of cranial internal temperature of 0.01° to 0.02° C.

(2) The metabolic response to cold, elicited by increased firing-rates of cold receptors of the skin which are subject to control from the "thermostat" by warm-impulses producing a strong inhibition (10 percent of a normal metabolic rate for an increase of central temperature by 0.01° to 0.02° C.) which becomes complete at the "setpoint."

These results are consistent with the accepted findings of experimental neurosurgery and electrophysiology obtained between 1885 (discovery of the anterior

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hypothalamic heat-loss center) and the recent observations by T. Nakayama, of temperature-dependent action potentials of the cerebral warm-sensor, which followed only 2 years after the gradient calorimeter had demonstrated the absence of peripheral warm-inflow to this site and its independence as a terminal sensory organ for warm-reception. The "temperature-eye" is analogous to the anatomically related retina (sensor for light), the thirst-center (osmotic sensor), the hunger and satiation-centers (sensors for biochemical blood composition), and the respiratory centers (p_H or HCO_3^--sensors) in the brain stem.

Microcalorimetry and Biochemical Thermodynamics

Work at NMRI on microcalorimetry was planned with two main scientific objectives:

(1) Among those characteristics of chemical change which may be exploited for the quantitative analysis of chemical or biochemical reactions, the liberation or absorption of heat is perhaps the only one not restricted to a certain type, group, or sampling of reactions. Continuously recording microcalorimetry may therefore determine the presence or absence of chemical interactions for which there exists no other analytical method. Initial and final rates, total extent, and often the reversibility and equilibrium state of such a reaction may be found by microcalorimetry. This makes the measurement of heat a widely applicable analytical technique.

(2) Two thermodynamic quantities, namely, the heat of reaction, $\triangle H$, directly measurable by calorimetry, and the entropy-change at finite temperature, $T\triangle S$, indirectly measurable with the microcalorimeter, are the driving forces of chemical change. The heat change, $\triangle H$, represents the tendency of chemical energy to be released and dissipated as molecular agitation. The entropy change, $T\triangle S$, reflects in a quantitative manner the tendency of matter to assume its most random, least orderly configuration and distribution in space. Measurement of heat and entropy-change are therefore essential.

Microcalorimetry has been restricted in the past to a few, specially equipped laboratories. Quantities of heat, to be measured by combustion-calorimetry ha had to be large, in the range of gram-calories. He pulse microcalorimetry (88, 100) developed at NMR makes the measurement of reaction-heats one tho sand times smaller a routine laboratory procedur Contrary to the usual approach in classical micr calorimetry, the new method does not attempt to isola and to preserve the difference in temperature, caus by a chemical reaction. Instead the heat is discharg at maximal velocity into a sink, through a thermopi of approximately 10,000 junctions. These are a ranged in parallel with respect to heatflow and series for their electric potentials. Reactants a spread out over a maximal surface area (approximate 10,000 square millimeters) in contact with the "ar thermopile." Mixing is performed by rotating mov ments of the entire instrument without disturbing m chanical devices. Reactions using fractions of a micr mole, with 1/20,000 molar solutions, or with a fe thousandths of a small calorie total heat change ma be investigated. A theoretical treatment, based on el mentary thermodynamic reasoning (101), has mad it possible to derive not only heat of reaction but als free energy and entropy changes, from two measur ments with the heat pulse microcalorimeter. Reven ibility has been demonstrated, and a complete therm dynamic analysis has been performed with glutamin hydrolysis, a reaction that is irreversible for all practica purposes (89).

Applications of the instrument at NMRI have re sulted in the first reliable determinations of the hea free energy and entropy changes of ATP hydrolys (102), in the first direct determination of the hea of a polynucleic acid interaction (103) as an exampl of driving forces in molecular events in genetics, an the first determination of the heat of an antigen-anti body interaction (1496). Objectives under immediat consideration are the oxidoreductions of the dipho phopyridine-nucleotide system, the indispensabl source of energy for synthesis of ATP in oxidativ phosphorylation, and for the assimilation of carbo dioxide in photosynthesis. These transformations o energy provide the driving forces for the entire proces of life.

VII. PHYSICAL BIOCHEMISTRY

S. L. Friess and R. F. Steiner

Introduction

For more than a decade, NMRI has pursued a vigorous program of fundamental research into the mechanisms underlying biological activities of nerve, muscle, and collagenous tissues. The relative emphasis on functional relations in these respective classes of tissues has shifted markedly during this time, in direct coupling with the ebb and flow of principal investigators in this disciplinary area. The orientation for research and the methods of attack on basic problems have been geared in general to produce fundamental information on how these tissues and their subcomponents operate both under normal conditions and under imposed stresses. Then, as each phase of study culminated in better understanding of a given aspect of subcellular structure or function, the results were scrutinized in terms of their applicability toward solution of long-range operational problems, particularly as related to the human factors element in newly-evolving naval weapons systems.

The research techniques employed in the bulk of the work to be surveyed have been both physical and biochemical. Starting at the lower levels of biological complexity, interest in mechanistic events attending the actions of isolated enzyme systems from mammalian muscle, mammalian and amphibian nerve, and assorted types of collagen, has led to the development and use of highly precise enzyme-kinetic techniques, and to the employment of sophisticated physical tools (light-scattering, ultracentrifugation, viscometry, etc.) for characterizing the sizes and shapes of enzymes and related macromolecules in solution.

At the next higher stage of organization, tissue organelles such as myosin threads, muscle fibers, single nerve fibers, and isolated nodal regions have been studied experimentally with respect to their biochemical properties in aggregate, and their excitation parameters (contractility, electrical impulse conduction) associated with physiological function. At this same level of organizational complexity, theoretical studies have been made on the statistical mechanics of muscle fiber models, with particular reference to processes involved in the transfer of chemical free energy into contractile work.

Finally, at the highest levels of biological complexity represented structurally by small intact laboratory mammals, amphibia, and neuromuscular preparations derived from these species, a variety of chemo-pharmacological, biophysical, and toxicological techniques have been applied to unravelling details of processes in which chemical agents react with tissue-localized chemoreceptor units to alter their physiological response to stimuli, in either a positive (response amplification) or negative (response blockade) direction. Much of this work incorporates contributions from other scientific disciplines, and has also served as a Navy inhouse bridge between certain NMRI research interests and operationally motivated programs sponsored by the Office of Naval Research. And finally some of the current research on basic mechanisms involved in amplification of neuromuscular response patterns shows direct promise of application to human responses under stress conditions imposed by deployment of current and projected weapons systems of the Polaris type.

Research Programs and Key Results

Muscle and its derivatives.—A highly-coordinated and intensive program on basic mechanisms involved

in muscle contractility, largely in the hands of Morales, Botts, Blum, Hill and their colleagues (1162), culminated in the early fifties in their "binding-deformation" theory of muscle action and its detailed steps germane to efficient work production. This theory is still current, and has stimulated much research on an international scale dedicated to confirmation or rebuttal of its main provisions.

In essence, this theory pictures a primary interaction between triggering adenosine triphosphate ions (ATP) and the oriented collection of macromolecules in muscle fibrils grouped under the title myosin (M) as an electrostatic adsorption at the fibril surface accompanied by simultaneous deformation of the fibril.

$$aM \text{ (rest state)} + b \text{ ATP} \rightarrow aM.bATP \text{ (deformed)}$$

This deformation on adsorption of the polynegative ATP species to the macromolecular, charged surface M produces a deformation as a result of the net neutralization of positive charge on the M surface through progressive electrical pairing of such surface groups as $-COOCa^{+}$ with negative ATP species.

Following this "binding deformation" step, the controlled enzymatic action of the endogenous myosin in splitting its bound substrate ATP and desorbing the products.

$$\underset{\text{(deformed)}}{aM.bATP} \longrightarrow bADP + bP_i + \underset{\text{(rest state)}}{aM}$$

results in regeneration of the surface charge on M, and ultimate restoration of both the macromolecular surface to its initial conformation and the fibril to its resting state.

This scheme, with its key step of triggered electrostatic binding of ATP species as the act directly responsible for fibril deformation and muscular performance of work, is in accord with the critical finding that Mg^{++} ion characteristically enhances the adsorption and binding of ATP and certain of its analogs, and is also essential for the overall contraction-relaxation process. Further, the controlling step of binding-deformation in this scheme is attractive in that the free energy changes associated with such ion-macromolecule electrostatic interactions account for large fractions of the net free energy of dephosphorylation inherent in the ATP-splitting step.

The extent to which this electrostatic binding model for muscle contractility approaches reality received a considerable boost from the statistical mechanical analysis of model contractile systems carried out by Hill (727) and colleagues. In this work, an assortment of elastic filament models was surveyed theoretically to find out whether considerable changes in length (e.g. at constant tension) might be expected as a function of change in surface charge on a given fiber. These statistical models portray the contractility potential of a fiber as a sensitive function of its charge distribution, independent of how that charge is produced, and therefore apply generally to such electrostatic binding models as that postulated by Morales and Botts (1162). In brief, such models of a fiber anchored at both ends and surveyed for length-tension relationships as surface charge is varied, show a phase-change region which accounts for the elastic properties of fibrils. In the critical phase-change region, a very small change in the molecular environment can cause a large and sudden contraction of the elastic element. Return to the initial state of extension of the fiber by a reversal of the initial change in surface charge is likewise prompt.

Finally, it might be noted that this electrostatic mechanism governing the state of extension of muscle fibrils, and ultimately the velocities of change in state as a result of a neural stimulus, had the characteristics required for operational control of muscular work by special chemicals administered to man (or animals). In a military context, it is conceivable that controlled intake of ATP-like agents by personnel in high-stress situations and confined atmospheres, via the aerosol inhalation route for example, might result in dose-controlled mediation of the intensities and amplitudes of muscular activity. Such responses even if small might well improve sustained performance in extended missions with high muscular demands.

Collagen and its derivatives.—A significant highlight in past biochemical research activities was provided during a 3-year period of residence of Lt. P. H. von Hippel, MSC, USNR. Dr. von Hippel added some very important elements to the muscle biochemistry program but this was overshadowed by the brilliance of his subsequent efforts in study of the biophysical chemistry of macromolecules from collagen.

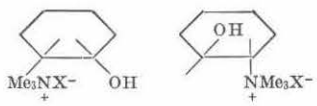
The scope and power of the experimental techniques employed by von Hippel and his colleagues in their study of collagen structure and function is illustrated by work on the purified fish collagen, ichthyocol (1696). This macromolecular constituent of connective tissue was subjected to collagenase-catalyzed degradation, and the kinetics of the proteolysis followed by two independent analytical methods (pH-stat and colorimetric ninhydrin). The kinetic analysis revealed

that, below the critical solution temperature (27° C.) at which collagen is transformed quantitatively into gelatin, there are local differences in polypeptide chain configuration in the vicinity of the susceptible peptide bonds, leading to marked differences in the rates at which these bonds are attacked and cleaved at a catalytic surface. This phenomenon was probed further by examination of collagen's molecular properties in solution, using viscometry, optical rotation, and light-scattering as the prime tools in evaluating structural transitions and molecular dimensions. It was found that the collagen macromolecule in solution can best be described as a rigid, multi-stranded structure with many hydrogen bonds linking the chains in the array of strands. As a consequence of this structure, which is essentially that of a "particle" in character, the enzymatic cleavage of single strands at points of high reactivity leaves the particle itself relatively intact and unaltered with respect to molecular weight, but brings about a partial structural collapse by introducing points of increased flexibility.

The results of this phase of von Hippel's program are of far-ranging significance. The finding that proteolytic enzymes can distinguish between loci of order and disorder along a protein chain, or perhaps between regions of "amorphous" vs. "crystalline" character in a macromolecule, has a powerful bearing on the rates of in vivo enzymatic reactions, and on the physiological functions which they in turn control. The disposition of configurations in natural protein chains into classes of order and disorder may be a major key to the mechanisms behind the turnover of structural or functional building blocks in mammalian tissues, and hence into the net balance between protein synthesis and destruction. Further, the demonstration that collagen structures are specifically attacked by proteases may offer direct insight into the modes of generation of collagen defects in man. Such defects, whether genetically controlled or induced by insults from the environment, may ultimately be managed by direct alterations (chemically produced) of the crystalline loci in the collagen aggregates which display the high sensitivity to protease attack.

Chemoreceptors in nerve, neuromuscular preparations, and their derivatives.—An ongoing program of research on basic mechanisms underlying excitation and conduction processes in medullated nerve, synaptic transmission at the mammalian neuromuscular junction, and processes of amplification or blockade of muscular response to neural stimuli has gathered momentum and scope during this decade, largely in the hands of Friess, Thron, Standaert, and their colleagues. Primary emphasis has been placed on organic syntheses of new, stereochemically tailored aminoesters based on such cyclic aminoalcohol structures as *cis* and *trans*-2-dimethylaminocyclohexanol, tropine and pseudo tropine, to furnish a series of molecular probes into the surface structure of the chemoreceptor loci responsible for excitation phenomena in neuromuscular tissues. These substances were then employed in high stereochemical purity to interact with the single fiber and isolated single node of Ranvier in frog sciatic nerve, the isolated phrenic nerve-diaphragm preparation from the rat, isolated muscular tissues, and even intact laboratory animals (mouse and cat, intravenous administration). Additionally, the aminoester probes and certain of their derivatives were used (as substrates or inhibitors) to examine fine structure in and about the catalytic site on the purified nerve enzyme acetylcholinesterase (AChE), in efforts to assess the function and importance of this enzyme in events attending nervous excitation.

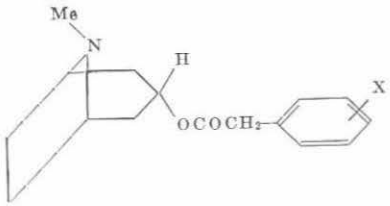
A notable advance in recent stages of the work (1470) was provided by the finding that the stereoisomeric aminoalcohols IC and IT evoke a



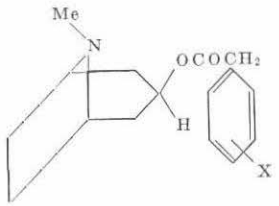
IC (cis) IT (trans)

complex spectrum of activities from the mammalian phrenic nerve-diaphragm preparation, starting with adsorption at the neuromuscular synapse from very dilute bathing solutions and the production of amplification of the twitch response height. Then, as the concentration of either agent in the tissue bath is raised beyond the initial 10^{-5}–10^{-4} M. levels causing response potentiation, the initial amplification of twitch response height is swamped out, and superceded by an overriding blockade of twitch response that can be reversed by drug removal and washing. Further, the dose-response relationship controlled by synaptic receptors is highly sensitive to the stereochemistry (*cis* or *trans* configuration) of the triggering adduct, with *cis* structures more potent by a factor of about 10 than their *trans* isomers, and with mixtures of the two triggering molecules evoking synergism (or exaltation) in muscular responses to stimuli.

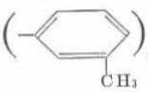
Even more striking in this biphasic response picture of twitch amplification-twitch blockade as a function of incubating concentration has been the behavior of newly synthesized aryl esters in the tropine and ψ-tropine series (1584).



Tropine Esters (trans)



ψ-Tropine Esters (cis)

These esters are particularly dramatic in terms of their power to produce highly amplified twitch responses via direct (muscle) or indirect (nerve) stimulation pathways, followed by abrupt decay of this amplitude potentiation into blockade over a very small range of incubating concentrations. Most impressive on this score has been the ester tropine-*m*-tolylacetate  , whose potentiating power is such that it can produce as high as an 80 percent amplification in twitch response from the diaphragm muscle, over a controlled time interval and in thoroughly reversible fashion.

These findings with the peripheral neuromuscular preparation open a rather promising avenue of approach toward solution of an important family of operationally oriented, human factors problems. These problems stem from the environmental stresses imposed on fleet personnel as a function of their deployment in the newer naval weapons systems, exemplified by the Polaris submarine on extended patrol. Prolonged submergence and exposure to sealed environments, accompanied by repetition of complex manual tasks, may well lead to loss of efficiency in muscular performance as a direct result of minor decay in amplitude or frequency of the motor nerve signals relayed from the central nervous system. Such decreases can be combatted either centrally, by psychic energizers, or peripherally at the end-effector tissues themselves. In the latter approach, the controlled administration of graded amounts of an ester like tropine-*m*-tolylacetate, via the aerosol inhalation or oral routes, may furnish an amplification factor in peripheral neuromuscular triggering events just sufficient to negate the results of decreased neural stimuli.

This attack on the problem of assuring small-amp physi tude precision in muscular movement is indeed ideal a ver suited for application in sealed atmospheres, since t the techniques of achieving and maintaining steady-sta form concentrations of the muscle-potentiating agents wit simu in operational personnel are simply reduced to fixi has the differential between easily controllable rates supply (via atmospheric aerosol) and rates of destru "bas tion or elimination from the body. Also, the rever tion bility in binding of these agents to chemorecepto beer ensures that control of muscular response in personn lem can cease promptly on leaving the operational enviro or ment, or on shutdown of the aerosol source.

The same aminoesters used so successfully in probi sten fundamental properties of the mammalian neuromu clin cular apparatus have also been employed in a bas alte study of nerve fiber conduction parameters, and and structural features characterizing the catalytic unit vie the nerve enzyme AChE (as obtained in high puri the from electric eel tissue). Some important generaliz in tions have emerged from this work. First, the tertia aminoesters do block excitation in the single node Ranvier in frog sciatic nerve fibers, with high specifici bel in terms of ester structure vs. potency, and with hig rib stereospecificity in terms of spatial orientation of pol na groups in isomeric pairs of esters. Similarly, the esters act as weak-to-moderate inhibitors of the AChE ag acetylcholine system, with high structural and ster st specificity evident in blocking potencies, and with *c* ch isomers in general more potent than their *trans* rela sc tives. But significantly, although there is a clear co fr relation between ester structure and ability to bloc te nervous excitation on the one hand, and between stru th ture and the ability to block the enzyme syste AChE-ACh in vitro on the other, there is no appare relationship in parallel between the two respectiv blocking processes as tested with the same set of prob ing agents. Indeed, strong elements of anticorrelatio occur. This finding, confirmed anew with each ne set of esters synthesized and tested, reduces sharply th likelihood that AChE localized in the nerve membran or axoplasm plays a large or key role in events attend ing the generation of the nerve impulse at excitatio loci.

Biopolymers.—The investigations pursued in thi highly important area of molecular biology have bee concerned with the structure and properties of th two most significant types of biological polymers, th proteins and nucleic acids. Intensive effort has bee directed toward precise evaluation of basic molecula properties, using techniques that are for the most par

physical in nature. Indeed, as this field is undergoing a very rapid expansion in the push toward unravelling the molecular basis for genetically controlled transformation and reproduction in living systems, the simultaneous development of new physical techniques has been essential to the prosecution of this research.

While the orientation of this research has been "basic," in the sense that a correlated body of information has been sought for its own sake, the results have been germane to the solution of Navy-oriented problems. Specifically, subtle changes in protein structure or function are intimately related to disease states stemming from these alterations, and can be used clinically as diagnostic aids. Further, the controlled alteration of macromolecular structure as in the viral and chromosomal polynucleotides is presently being viewed critically from the standpoint of projected synthesis of new biological warfare agents to be deployed in naval weapons systems.

Recent findings in this challenging field, at the hands of Dr. Steiner and his colleagues, are summarized below under the two groupings: (*A*) Biosynthetic ribonucleic acid; and (*B*) Fluorescence properties of natural and conjugated proteins.

A. Biosynthetic ribonucleic acid.—Ribonucleic acid (RNA) is now generally recognized to be the directive agent of protein synthesis. The genetic information stored in the deoxyribonucleic acid (DNA) of the chromosomes as specific nucleotide sequences is transcribed into complementary nucleotide sequences in freshly synthesized RNA. This in turn serves as a template for the guided assembly of amino acids into the polypeptide chains.

The central role of RNA in mediating protein synthesis appears to be universal. In the cases of certain viruses RNA is the only nucleic acid present and assumes responsibility for the storage of genetic information, the replication of the virus, and the synthesis of its protein subunits. A number of viruses of this class are known whose purified RNA has infectious properties. Such systems are of considerable clinical and military interest, in particular with regard to possible applications to bacteriological warfare.

A knowledge of the molecular organization of RNA is essential for an understanding of its biological activity. One approach to the problem has been to examine the properties of synthetic analogues of RNA, which can be prepared enzymatically with any desired nucleotide composition.

Synthetic polyribonucleotides have been prepared which contain only one of the four nucleotide bases present in natural RNA (1515). Such homopolymers can exist in either of two different molecular states, depending upon conditions. These correspond to an incompletely organized configuration in which much of the molecule is in a randomly coiled state, and to a highly organized helical structure.

In the case of polyadenylic acid (poly A) the transition from the unorganized to the helical state can be brought about by reducing the pH to a value below about 6, depending upon the ionic strength. At neutral or alkaline pH poly A exists as a partly organized system, containing alternate helical and amorphous zones (57, 1515). In the presence of high levels of methanol, all helical content is lost and the polymer reverts to the completely random state.

The transition between the unorganized and helical states is remarkably sharp, going to completion over about 0.1 pH units. In terms of current theories of the helix-coil transition, this indicates that the structural transition is initiated at relatively few points along the chain, the enlargement of an existing helical sequence being energetically favored over the formation of a new sequence.

The helix-coil transitions for other homopolymers, as well as for complexes of two different polynucleotides, have been examined and found in all cases to be rapidly reversible (1504). Thus no intrinsic kinetic barriers exist which prevent the exploration of competitive configurations by polynucleotides, and the selection of that most favored energetically.

Copolymers of two different nucleotides have been examined. In several cases these have been found to possess fractional helical contents by the criteria of ultraviolet hypochromism and optical rotation. As the sequence of nucleotides in these copolymers is entirely random, this result indicates that the formation of helical regions in mixed polynucleotides is not dependent upon any specific linear order of nucleotides.

The interaction of synthetic polyribonucleotides with the vital stain acridine orange has been studied (1502). The binding is accompanied by pronounced changes in the fluorescence intensity and adsorption spectrum. The character of these depends strongly upon the nature of the polynucleotide and upon whether it is in the helical or random state.

B. Fluorescence properties of natural and conjugated proteins.—Virtually all proteins are endowed with the property of fluorescing at ultraviolet wave lengths, by virtue of the presence of the aromatic residues tryptophan and tyrosine. Of the two, tryptophan, whose emission band is centered at about 3400–3500 Å, has

by far the strongest fluorescence and dominates the emission spectrum of the proteins in which it occurs.

The intensity of fluorescence of the tryptophan residues of proteins has been found to be very sensitive to the state of their molecular organization and can, in many instances, provide a means for monitoring any change in the configuration of the protein. In this manner, the intensity of tryptophan fluorescence has been developed as a structural probe for proteins and has been used to determine the kinetic parameters characterizing the denaturation of pepsinogen, lysozyme, and soy bean trypsin inhibitor (1516).

The intensity of tryptophan fluorescence is reduced by the binding of an ion or molecule whose absorption band overlaps the emission band of tryptophan. This is a consequence of radiationless energy exchange between the tryptophan and the bound molecule. This process is being used to estimate the binding of thyroxin by the plasma proteins involved in its transport.

The property of visible fluorescence may be conferred upon proteins by coupling them with a fluorescent dye, such as fluorescein isothiocyanate or 1-dimethylaminonapthalene-5-sulfonyl chloride (1518). Such visibly fluorescent conjugates are very useful for studies of the configuration and interaction of proteins.

Both the intensity of fluorescence of the conjugate and its degree of polarization are very responsive t changes in the molecular state of the labelled protein In particular, fluorescence polarization is a very sensi tive index of any loss of structural rigidity under de naturing conditions. The denaturation of γ-globuli and the thyroglobulin have been studied in this way Both proteins have been found to display all gradation of flexibility ranging from the relatively rigid state o the intact molecule to a configuration approachin that of a completely structureless coil.

An example of particular interest is the reversibl inactivation of pepsinogen. In 9M urea the com pletely reduced form of pepsinogen, all of whose di sulfide crosslinks have been split by treatment with a sulfhydryl reducing agent, approaches a structureles condition. Removal of the urea and reformation o the disulfide bridges by atmospheric oxidation, con verts almost 50 percent of the material to a form which is indistinguishable from native pepsinogen in physica properties, and which can be converted to active pepsin.

This result indicates that the complete molecula organization of pepsinogen appears to be dictated b its primary structure. This finding is of course o considerable interest for attempts at protein synthesis

VIII. BODY COMPOSITION AND PHYSICAL CHARACTERISTICS

Julius Sendroy, Jr.

Introduction

The assessment of man's ability to function, work, and to live under the conditions governing reaction between his body and that of his environment has become increasingly important, not only for achievement but for survival. The availability of criteria for the evaluation of an individual's capability and physical well-being, with particular application to military, industrial, and medical activities is of growing concern to our modern society.

Physical anthropology, through the medium of anthropometry and the measurement of body composition, has occupied the attention of biologists and physicians for the past half century. Interest and activity in the investigation of the possible relationships of physical characteristics and chemical composition to metabolism, physiological functions, reaction to drugs, susceptibility to disease, and personality patterns, have increased greatly during the last two decades. This interest is reflected by the mass of literature on studies of the type, build (structural or constitutional), size, volume, weight, height, density, surface area, fat, water, and lean tissue of the body and its components and/or compartments.

Indeed, from the time of Hippocrates (about 400 B.C.) body build has been more or less associated with certain types of disease as man was typologically classified under the heading of extremes, such as slender, corpulant, and stocky. More recent students of body build, such as Kretschmer, Conrad, and Sheldon, have developed and used theoretically based methods of measurement and classification of body types. On the other hand, strictly empirical, objective methods have been used by others for the informative and objective analysis of body build. In the studies of Lindegård, Keys and Brozek, Behnke, and many others, a range of physical factors or parameters of body build, based on variations in the quantities of different tissue components of the body, reflects not only the outer configuration, but also the structure of man's most intimate earthly dwelling.

In the operation of the Armed Forces, practical considerations make particularly important the employment of anthropometric and biometric techniques for the solution of problems of supply and logistics, and the deployment of personnel, space, and material. From its inception and throughout the 20 years of its existence, various members of the staff of the Naval Medical Research Institute have developed methods for, and conducted studies of, body characteristics (volume, surface area, specific gravity) and composition (fat, water, lean body mass). These will be reviewed in the following.

Body Composition

Specific gravity, total body fat and water.—In the course of mobilizing millions of men for the war effort in 1942, it rapidly became apparent that standard age, height, and weight tables were sometimes misleading as an index of physical fitness. Thus, the best physical types, exemplified by outstanding athletes, could easily be rejected as being overweight. A much more reliable index of obesity than that of absolute weight was found in the ratio of weight to volume (obtained according to the principle of Archimedes by weighing the body immersed in water). The resultant was a value for the average specific gravity of the body indica-

tive of the relative amounts of bone, fat, muscle, and watery tissue present. On this basis, owing to the low density of fat relative to other body tissue components, a much finer distinction could be made between the well-developed muscular physique and true obesity indicated by an abundance of fatty tissue (59, 1742).

With the foregoing as background, one of the earliest programs in the history of NMRI was one by Pace and his coworkers, involving an intensive theoretical and experimental study of body composition (1228, 1304). Equations were developed and practical methods described for the determination of body specific gravity of guinea pigs by water displacement, and the subsequent calculation of total body fat from the former. Since the values for specific gravity of men were found comparable to those for the (eviscerated) guinea pig, a similar relationship between fat and body density was established for the conversion of the latter to percent body fat in man (1146, 1228). Further work showed that the proportions of water and of elementary nitrogen content to body weight of guinea pigs were constant, provided body fat was taken into consideration. The available experimental evidence thus gave support to the concept of a lean body mass that is relatively constant in gross composition, in which body fat may be considered present as a diluent (1217).

Additional contributions toward the establishment of the experimental relationships of body specific gravity or density, body fat, and body water, and methods for their evaluation, were made by NMRI investigators (1, 1167). These results have in the main been repeatedly confirmed in other laboratories. It was also shown by an extension of the work to human subjects, that the factors of body composition could be related to those of body build (440, 441, 1208), such as the somatotypes developed by Sheldon.

Inert gas exchange in diving and air flight.—The work on the relationships of the various tissue components of the body, undertaken from the standpoint of body build and composition as an index of general health and of efficiency and fitness for carrying out tasks and duties, was applied in another practical direction of extreme importance for military and other air and underwater operations.

From the logical assumption based on animal experiments, that water consistently constitutes 72.7 percent of the lean body mass of man, the body may be considered in terms of two components, one fat, and the other aqueous. Gaseous nitrogen, the major component of air, is dissolved in each of these in accordance with the extent of its solubility in each. It was shown that body composition in terms of fat and water may be quantitatively estimated from their inert gas solubilities (1152, 1217, 1228). Moreover, since fat dissolves five times as much nitrogen as does water, the retention of the gas in the tissues with subsequent release when the pressure is lowered, as when an aviator rises to altitude in the open or a diver surfaces from depth, gives rise to "decompression illness," or the "bends" and "chokes." These are characterized by major signs of pain, asphyxia, and paralysis, and minor signs of pruritis and skin rash.

The individual adaptation to variation in the atmospheric pressure of the environment is of major importance in diving and high altitude operations where today men descend to watery depths of 750 feet and ascend to altitudes of over 240,000 feet. The nature of the inert gas exchange characteristic of the individual will determine his ability to adapt to and carry out his work and mission under such conditions. Therefore, a knowledge of the principles of physiological inert gas exchange and the factors governing it, such as the solubility and diffusion of gases in and through various body fluids and tissues, with variations in temperature and pressure, are of prime importance.

These problems were keenly appreciated by Behnke (74), who, shortly before coming to NMRI, had personally participated in the operations of the rescue of the crew and of the salvage of the sunken submarine "Squalus." With the onset of war, research on related projects inevitably occupied the attention of NMRI workers, who proceeded along two fronts of attack. Theoretical background studies by Smith and Morales on inert gas exchange as a physical system (1149, 1150, 1152, 1435, 1436) and a series of laboratory investigations by Gersh and Catchpole and their coworkers (217, 622) were carried out. In the latter studies, the physical and physiological factors affecting the formation and appearance of gas bubbles in blood and in the tissues of animals of several species, under various conditions of atmospheric compression and decompression, at high and low pressures were studied. This work was summarized in reviews which encompassed such subjects as the mathematical principles involved and the physical factors responsible for bubble formation and gas uptake and elimination, the formation and accumulation of gas nuclei in the body, the rate of decompression (pressure release), gas solubilities, body composition, the flow of blood in the tissues, and the amount (density and surface) of blood capillaries present (74, 217).

These studies provided increasingly greater evidence to show that gas bubbles are primarily responsible for causing the pathological effects of decompression sickness, and that under all circumstances such bubbles are mainly within the blood vessels (aeroembolism), and account for nearly all of the important signs and manifestations of the decompression sickness symptom complex. Bubbles in tissues other than the blood are restricted to tissues rich in fatty material and occur under certain severe conditions of decompression from high pressures.

The pathological effects experienced by divers in decompression from depth were found to be much greater than those of air personnel ascending to altitude, owing to the greater bubble frequency (number) and distribution in the former case. The relatively small amount of gas in solution at altitude causes relatively fewer neurological signs of damage to the brain as compared with the consequences of aeroembolism from deep diving. The rarity of spinal cord involvement in altitude decompression may also be owing in part to the usually immediate recompression from altitude to ground level on the first appearance of symptoms. Such relief is also much more rapid because of the greater water and carbon dioxide content of the altitude bubbles as well as their smaller number, compared with those which arise in tissues on decrease of pressure after exposure to diving depths.

The possibility of effectively diminishing the incidence of decompression sickness in divers and aviators was suggested by the results of experiments on human subjects. By the addition of carbon dioxide to oxygen inhaled following exposure to pressure, the rate of nitrogen gas elimination from the body was increased 20 percent during the first 30 minutes of denitrogenation (1025).

Zoometry and population stress.—In a series of observations of a different nature, the effect of another type of stress on body measurements in animals was studied by Christian. Of significance, in the possibility of a parallel in anthropometry, were his findings of various correlations between adrenal weights and body weights for several species of animals and man, body adrenal gland and reproductive organ weights, and population size or "social rank" in mice. A decline in reproductive function and an increase in adrenocorticotrophic function were attributed to stress resulting from increasing population pressures, with increasing population size (238, 241, 376) (see ch. VIII).

Body Surface Area

The surface area of the body, total, or of its parts, has been found to be one of the most useful of all the physical dimensional characteristics of an animal. This measurement has been correlated with many other biological and physiological variable and indices, such as development and growth, oxygen consumption, basal metabolism, body heat production, lung carbon monoxide diffusing capacity, extracellular body fluid, lean body mass, and body volume. It has been applied with varying degrees of success in the treatment of burn cases and radiation effects, for the assessment of drug dosage and body covering, and has been involved in the approach to aircraft engineering design by studies of the realtionship of aircrew movement and performance in limited spaces (915, 1674).

On the other hand, despite its wide applicability and use, this physical measurement is probably the most difficult to make directly. Historically, its use as an anthropometric variable was preceded only by those of weight and height, with which it has repeatedly been related both on rational and experimental grounds. Following Abernethy's first estimate in 1793, a succession of techniques of direct physical measurement was evolved, of a cumbersome and impractical nature.

The introduction of mathematical techniques to direct surface area measurements led to the development of geometric methods, whereby surface area was estimated by assuming that parts of the body more or less resemble regular geometric solids. Various types of formulae were developed, theoretical and empirical, for calculating surface away from its geometric relations to the major dimensions (height and weight) of the body. Most of these formulae for the indirect estimation of surface area in both categories were based on insufficient data and consequently gave reliable results only within a narrow range of human physiques (i.e., for the "average" man). With the passage of time, many of these methods of calculation have been discarded.

A noteworthy exception has been the theoretically based, well-known DuBois and DuBois height-weight equation which has been used more extensively than any other. Because the calculations are practical only in logarithmic form, many tables and charts have been constructed and devised to overcome the difficulties of the calculation. In the empirical group of calculations, the basic procedure is to plot the surface area value against one or each of the other dimensions, and then to find the average curve of the points by graphical

or algebraic methods. The self-adjusting power equation of Boyd developed in 1935, in which the exponent of weight is automatically decreased as the weight itself increases, is probably the most sophisticated of this group of empirical equations and justifies her claim to "the best single equation for predicting surface area from other dimensions of the body."

One may say that with the exception of another method, the photographic, no new developments occurred from the time of Boyd's masterful treatise of 1935, until a few years ago. New and improved techniques and approaches to the study of body composition, such as have been cited in the foregoing, spurred a revival and a widening of interest in the relationship of surface area to other body measurements.

Owing to the situation outlined, there arose in the author's laboratory, an effort to develop a simple, rapid, and accurate method of calculating human body surface area from other simple body dimensions. From the empirical relationships of the sum of height and weight, and the "shape" factor of the ratio of weight to height, a diagram was constructed whereby surface areas may be obtained conveniently and accurately, from values of height and weight alone (1390). A comparative evaluation and statistical analysis of the method applied to 252 measurements of surface area from 0.05 to 3.0 m^2, indicated a margin of superiority for the graphical method in respect to accuracy especially in the case of abnormal body types, as compared with the well-known DuBois height-weight formula. A consideration of the rationale of the anthropometric relationships involved (from the prenatal to the "giant" size), indicates that they are in accord with accepted concepts pertaining to the growth and development of the human body.

Subsequently, a convenient and rapid photographic technique (1386) of obtaining data which can be used for the calculation of human body surface area was described (1395). The results, which were in good agreement with values obtained by a reliable method of readings from a chart, provided additional support for the application of the increasingly important photographic method of quantitation in human biology. Data were also obtained which suggest that the surface area of dogs may satisfactorily be estimated, with a modification in calculation, by the same previously reported chart method used for human beings (1395). In the same paper, empirical equations for the calculation of body volume (and density) in man based essentially on measurements of weight and height were developed and tested in respect to measured values obtainable from the literature. The statistica evaluation and the criteria of convenience and rapidit in use, rather than more restrictive theoretical con siderations, indicate the superiority of predominantl empirical relationships as the methods of choice fo the prediction of body volume. Comparison of th reliability of the results with those obtainable by estab lished methods of quantitation indicates that thes equations may be useful as approximate, but mos convenient indices of gross body composition (1396)

As an alternative to the diagram used in the graph ical calculation of human body surface area fron height and weight according to the method of Sendro and Cecchini, a line chart or nomogram was con structed (1398). The relationships involved hav been published in the form of tables indicating surfac area for known height and weight in man in tw biological handbooks (1399, 1403) and in many text and monographs covering various phases of biolog and physiology.

Summary

Salient contributions have been made by investiga tors of NMRI in the field of body composition and physical characteristics as follows.

A more reliable index of physical fitness, or index of obesity, was developed in the use of body density rather than weight. This was followed by the concept of lean body mass, which has been widely accepted and proven most useful. The associated value of 72 percent water in lean body weight may be regarded as an important biological constant. Studies of inert gas exchange have contributed to better understanding of the phenomena of "bends" in aviation and underwater operations. Habitability studies in respect to popula tion density have shown the relationship of body size and composition to existence in close quarters. New, more reliable methods of arriving at a value for the body surface area of human beings (and dogs) have been developed to provide a replacement, with ex tended application in range of body size, of methods in use during the previous four or five decades.

The acceptance, recognition, and use by other work ers of the concepts advanced and the means and meth ods developed for their use, are an indication of their value as outstanding contributions to physical anthro pology and anthropometry. They represent, therefore, answers and solutions to important problems, not only in naval operations, but also more generally, in activi ties involving industry and public health.

IX. ENDOCRINOLOGY

J. J. Christian

Research in endocrinology at NMRI began early in 1947 with the investigations of E. P. Vollmer. There was also a significant amount of endocrine research by investigators from other branches of physiology. Collaboration in particular programs by personnel of various administrative divisions often made research possible that otherwise could not have been carried out. Notable in this respect was the opportunity provided for Nelson, Hume, Egdahl, and Richards to carry out a sustained collaborative program on the effects of various environmental and physiological stimuli on pituitary-adrenocortical function. Endocrine research largely centered around adrenocortical physiology and the role of the cortical hormones in the pathogenesis of, or resistance to, radiation damage, infection, potassium toxicity and diabetes or pituitary adrenocortical responses to environmental factors such as cold, heat, elevated atmospheric CO_2, crowding and similar factors pertinent to the problems of the naval service. Such practical problems required basic research which was in itself remunerative from both applied and theoretical points of view. Conversely, some basically conceived research had widespread application in medicine and human ecology, including military. There also was some research in reproductive endocrinology, most of which was ancillary to, or involved in, pituitary-adrenocortical research. In reviewing the history of these problems, one is impressed by the faithfulness with which they reflected progress and development of the field as a whole. In addition, a number of investigators who came to NMRI for 2-year tours of duty contributed importantly to the research and gained collaborative experience and contacts which had a lasting impact on endocrinology.

It is appropriate in reviewing endocrine research at NMRI to discuss it by programs rather than to present a strictly chronological sequence.

The early investigations of Vollmer, beginning 1947, were on the role of adrenocortical hormones in resistance to infections. There was a widespread belief at that time that cortical hormones conferred resistance to the effects of infectious disease, based largely on the well-known vulnerability of adrenalectomized animals and man to infection, and the similarity between the effects of adrenalectomy and many symptoms of shock, radiation injury, disease, and other detrimental stimuli. Related to this program was the problem of the Waterhouse-Friderichsen Syndrome and its prevention in acute massive infections. A survey of the literature at that time indicated that only those patients survived who had received some sort of hormonal support. However, lacking pure cortical hormones, a separation could not be made between the need for corticoids in general metabolic well-being and their specific effects on mechanisms of host resistance. In the middle and late 1940's several reports were in existence claiming that adrenal extracts, when given at the same time as antigens, enhanced antibody formation. Vollmer's experiments indicated that adrenal cortical extract (ACE) (cortisone and hydrocortisone were not available then and practical methods for measuring cortical hormones in biological fluids had not been developed) increased survival of mice with light, but not with heavy pneumococcal infection (1676). However, ACE in large doses had no effect on immunization with pneumococcus vaccine as shown by subsequent challenge with living organisms (1679). When pneumococcal infections in mice were attenuated by sulfadiazine, ACE had no effect on final mortality, although

it did prolong survival time (1681). The concept adrenocortical exhaustion following prolonged hyperstimulation, based largely on morphological studies, was widely accepted in the late 1940's and early 1950's. In this context, experiments with pneumococcal infections led to the inference that severe infection might result in early and rapid adrenocortical depletion and exhaustion which were only partially alleviated by ACE (1680). These experiments were addressed to the problem of infectious disease in naval personnel with the aims of improving methods of treatment, preventing mortality in the Waterhouse-Friderichsen Syndrome, and exploring the relationship of the adrenal cortex to infectious disease in general. The ideas of that time are now largely of historical interest since the availability of cortisone by 1950 and of other pure corticoids later, together with the development of methods for directly measuring adrenocortical hormones, almost reversed these earlier theories regarding the role of corticoids in infection. This change was first reflected at NMRI by experiments showing that growth-inhibiting doses of cortisone increased mortality in mice from Japanese B encephalitis, but that low doses had little effect (1682). Subsequently it was shown that cortisone markedly enhanced Coxsackie infection in adult mice (175). These results were consistent with a large amount of work of others on similar problems. From these results it was assumed that stimuli which increased endogenous corticoid production should decrease host resistance and enhance infection. Again using Coxsackie infection in adult mice, it was found that cold exposure had the same effect as cortisone, but that elevated temperatures did not enhance the disease (176). It is now clear that the cold increased glucocorticoid secretion and thus decreased host resistance, whereas heat probably increased aldosterone and not glucocorticoid secretion. Thyroid hormones may also have been involved, as their secretion is increased by cold and depressed by heat. Finally, it was shown that crowding, as a stimulus to increased adrenocortical activity, depressed inflammation and experimental granuloma formation in mice (249).

These experiments clearly demonstrated that glucocorticoids have an adverse effect on the normal mechanisms of defense against infection and on the reparative processes. The clinical implications are obvious and now well known. Earlier work with adrenocortical extracts suggesting beneficial effects are probably explainable by the presence of other hormones in the extracts.

Beginning in the 1930's there was a school of thought which attributed many of the effects of disease and tissue damage as well as one of the causes of shock to excess potassium. This arose in part from the similarity of these conditions to the effects of adrenalectomy with its high serum potassium levels. One of the principal proponents of the idea was R. L. Zwemer who in the late 1940's recommended his investigations on potassium toxicity at NMRI. He had earlier shown that adrenocortical hormones protected against potassium toxicity. The importance of this program to the Navy was in the possibility that potassium poisoning following its release from damaged tissues might explain some of the deleterious effects of radiation and might be important in the genesis and treatment of shock. The uniformity of response to potassium also suggested its use as an experimental tool to evaluate factors in host resistance. With this apporach an active collaboration began between Vollmer and Zwemer.

Tolerance to potassium and symptoms of potassium poisoning are remarkably uniform and unlike many other toxins, it is toxic in amounts in relation to body weight for a wide variety of vertebrate species (1796). Glutathione (GSH) was known to decrease the lethal effects of radiation and to protect against alloxan diabetes. Also, it is abundant in the adrenal cortex and lowers adrenal ascorbic acid. Therefore, it was thought that the adrenal cortex might be involved in the protective effects of GSH. This compound was found to have a marked protective effect against potassium toxicity which appeared in large part not to be mediated by the adrenal cortex (1797). Nevertheless it was concluded that at least part of its protective effect stemmed from increased adrenocortical activity since it reduced adrenal ascorbic acid and cholesterol (207). The question presumably was settled by showing that GSH had the same protective effect against potassium poisoning in adrenalectomized mice (1033), and that mice satisfactorily tolerated parenteral doses of GSH well above those used to confer protection against potassium (1683). Oxidized and reduced GSH increased blood sulfhydryl (SH) levels and both protected against potassium toxicity. Therefore the protective effect of GSH could have been due to increased blood SH levels. However, the rates of decline of SH levels and protective action were not the same so that SH levels were not directly related to the protective effect of GSH (1684). This lack of parallelism necessitated consideration of other mechanisms. Interference with blood respiratory processes seemed possible, especially as GSH and SH are intraerythrocytic. Methemoglobin production was used to disturb blood respiratory processes and the relationships between

its production and blood levels of GSH and SH were studied (1688). Paraminopropiophenone (PAPP) and sodium nitrite were selected as methemoglobin producing agents, because both confered protection against radiation injury and cyanide poisoning; also PAPP produced methemoglobin indirectly and sodium nitrite directly. Both compounds elevated SH levels appreciably and lowered GSH slightly in vivo, but only sodium nitrite had these actions in vitro. There was some parallelism between methemoglobin formation and SH levels, and in the absence of methemoglobin formation the changes in GSH and SH did not occur. Thus these changes occurred in vitro and in vivo in association with the direct production of methemoglobin by sodium nitrite, but only in vivo with the indirectly acting agent, whereas blood GSH levels were lowered and SH unaffected by alloxan which is a poor producer of methemoglobin. These experiments also demonstrated that appreciable increments in blood SH were compatible with survival. These results were of particular pertinence to naval medicine because of numerous reports implicating SH compounds in one or another aspect of shock, radiation injury, or response to other harmful agents. Based on reports that GSH protected against alloxan diabetes, that SH was involved in the pathogenesis of diabetes, and other pertinent evidence, additional experiments were completed in which it was shown that both PAPP and sodium nitrite protected against alloxan diabetes (1687). Continuing this same line of reasoning and in view of the fact that prolonged hypercorticalism resulted in diabetes, the effects of cortisone and ACTH on blood SH levels were explored (1686). However, neither substance had a consistent effect on blood SH levels. In a review (1689) Vollmer concluded that few, if any, fundamental relationships between endocrine function on disease and SH levels had been proved. At the same time, it was realized that a heterogeneous variety of agents were similarly protective against alloxan diabetes, but that all they had in common was a marked generalized toxicity at protective doses, thus suggesting a common nonspecific mode of action via their toxicity, possibly the release of epinephrine and norepinephrine from the adrenal medulla. Indeed, epinephrine, but not norepinephrine, was found to have a striking protective action against alloxan diabetes which was unrelated to a concomitant hyperglycemia. Therefore the various protective agents may have produced their effects through the release of epinephrine. The mechanism of action of epinephrine in this regard was unknown, although a vasomotor component may have been involved (1691).

Thus a program which had been started in order to investigate the role of the adrenal cortex and its possible protective action against harmful agents circuitously returned to the adrenal, but to the medulla rather than the cortex. In following this devious but logical path much insight was gained into protective mechanisms, and many hypothetically protective substances or mechanisms were systematically proscribed—a necessary and important function of research. Also the basic problem became more clearly defined and avenues for further investigation suggested, although no panacea for radiation injury evolved.

Zwemer and others had long recognized the similarities between shock and potassium poisoning and suggested that shock might at least in part be the result of the release of potassium from damaged or hypoxic tissues into the extracellular fluids. Others had suggested on the basis of similar observation that the release of histamine might be important in the genesis of shock. Neither potassium nor histamine concentration in blood or extracellular fluids were found to be sufficiently high to produce toxic symptoms. However, the combined effects of the release of both of these substances from tissues might result in shock where neither one alone sufficed. In subsequent experiments it was demonstrated that simultaneously administered potassium and histamine acted synergistically, and not simply additively, in producing toxic phenomena and mortality (1798). Addition of a small amount of one of these substances greatly enhanced the toxicity of the other. The synergism can not be explained by the ability of histamine to increase blood potassium levels, as it does not have this effect in all species. Nevertheless, it was concluded that simultaneous release of histamine and potassium from traumatized or hypoxic tissue into the general circulation could explain effects beyond those attributable to either substance alone.

Radiation injury in many ways suggested altered adrenocortical function and thus led to interest in adrenal cortex. Early experiments on the role of the adrenal cortex in radiation injury and mortality depended largely on a variety of indirect methods for assessing adrenocortical function, as direct methods for measuring cortical secretion were not available. In 1949 the Radiation Division of NMRI used urinary 17-ketosteroids to assess adrenocortical function more directly following irradiation (953). Radiation was followed by a significant rise in 17-ketosteroids in dogs, indicating increased adrenocortical secretion. Subsequently it was shown that adrenalectomy increased

the sensitivity of mice to irradiation and markedly increased mortality (336). These investigators also demonstrated, as mentioned earlier, that glutathione had some protective action against radiation mortality. F. Ellinger found that cortisone in rather high doses increased mortality from radiation (477) and also carried out a number of other studies as well on relationships between steroid hormones and radiation damage (479).

Interest in adrenocortical function following irradiation continued beyond these earlier experiments and an extensive program was begun using perfused calf adrenals to investigate the function of irradiated adrenals and of adrenals from irradiated animals. This program started as a collaborative effort between the Endocrinology Branch of NMRI and the Worcester Foundation for Experimental Biology, headed by Gregory Pincus. The latter institute pioneered research in adrenocorticoid biosynthesis and developed the necessary techniques for adrenal perfusion and steroid analyses, while NMRI had the equipment and techniques needed for irradiation of the animals. Lt. Comdr. George Rosenfeld, MSC, USN, competently carried out the Navy's part of this program and was eventually responsible for a major part of the entire program. This research provided basic information on corticosteroid biosynthesis in irradiated and nonirradiated adrenals.

The first results to come from this program were published jointly by members of the staffs of NMRI and the Worcester Institute (1325). Radiosensitivity of the adrenal cortex was demonstrated by the appearance of a marked decrease in the secretion of corticoids by gamma-irradiated isolated perfused adrenals stimulated by ACTH. Reduced steroidogenesis reflected impaired activity of all of the involved enzymatic systems (1632). Concurrently, steroidogenesis in intact, nonirradiated perfused calf adrenals was studied using relatively simple experimental conditions in order to establish baselines and to validate procedures (1326). Oxygen was found to be necessary for steroidogenesis. ACTH increased the hydrocortisone to corticosterone ratio and in addition increased total steroid production and augmented the reduction of adrenal cholesterol and ascorbic acid. Ascorbic acid was not essential for steroidogenesis, and added GSH, as well as high levels of other substances normally found in blood, did not affect steroidogenesis. ATP was the only substance, aside from ACTH, which directs stimulating action. Perfused calf adrenals were found to secrete a sodium-retaining substance tentatively identified as aldosterone (1321, 1327). Secretion of this substance was unaffected by growth hormone, possibly increased by ACTH, and definitely increased by a lowered Na/K ratio in the perfusate (1332). Bacterial pyrogens in whole blood, but not in an artificial medium, inhibited steroidogenesis by general suppression of enzymatic activity, thus implying that some substance in whole blood was necessary for this effect of pyrogens (1328). Acetylcholine, and to a lesser degree methacholine, directly stimulated steroidogenesis in vitro, while carbachol, pilocarpine and nicotine had no effect. Increased adrenocortical secretion following splanchnic stimulation in some species thus may be due directly to acetylcholine (1329).

Using radiolabelled precursors, it was shown that the C20–21 side chain of corticoids was derived directly from the same side-chain on cholesterol (212). Studies with enzyme inhibition, such as amphenone B or irradiation, and aerobic and anaerobic perfusions showed that decreased adrenocortical metabolism was always associated with decreased steroidogenesis but not necessarily the converse, and that there may be a marked dissociation between metabolism and steroidogenesis (1330, 1334). Anaerobic perfusion resulted in decreased activity of the hydroxylases in steroidogenesis. Furthermore, adrenocortical tissue rapidly oxidized Meyerhof-Emden and Krebs-Johnson carbohydrate intermediates in contrast to amino and fatty acids. Studies with selective enzyme inhibition confirmed the role of glycolytic and terminal aerobic pathways in adrenocortical metabolism. Amphenone B was found to inhibit 11B, 17α and 21 hydroxylation and oxidation of the Δ^5-3B hydroxyl groups to the Δ4-3 ketone. In other words enzymatic reactions essential for steroidogenesis in the adrenal ester were blocked. A number of other inhibitors were studied that were less effective than amphenone (1333). A scheme derived from these and earlier studies was published for adrenal steroidogenesis by the calf adrenal.

Anterior pituitary hormones in addition to ACTH were tested for their ability to stimulate adrenal steroidogenesis, but none except ACTH had any effect (1335).

The desirability of using urinary steroids to assess adrenocortical function following radiation prompted a study of urinary excretion of labelled corticoids. However, it was found that biliary route was a major channel for excreting steroid metabolites by calves; so that urinary studies would have little value (1633).

Having obtained the basic information on steroidogenesis by perfused calf adrenals, attention was turned again to the effects of radiation on adrenocortical function. In these studies the animal received a single lethal dose of whole-body radiation in the NMRI

Co^{60} irradiator and the adrenals were subsequently removed at various time intervals and perfused. The general effects of a single lethal dose of gamma radiation on the animal and the technique of irradiating the calves were described (1337). At the same time it was shown that the steroidogenic capacity of the adrenal cortex increased within 24 hours after irradiation, declined to normal by the fourth day and rose again preterminally. The preterminal secretory capacity was appreciably less than that at 24 hours, and the secretion per gram of adrenal was reduced 35 percent, but steroid secretion per gland in terms of body weight was 25 percent above normal. The adrenals were markedly hypertrophied terminally with no evidence of cortical "dysgenesis" or "exhaustion" despite generalized sepsis, toxemia, hemorrhage, anemia, and other pathologic sequelae of irradiation (1336, 1338). Thus they were "holding their own" at death. From these results it was concluded that the concept of "adrenal exhaustion" following prolonged hyperstimulation might be misleading or erroneous. This concept had arisen largely from the morphological changes, including loss of lipid, associated with intense stimulation of the adrenal cortex. It is now known that active secretion is associated with a loss of intracellular lipid and other changes originally considered to be synonomous with "exhaustion." Thus Rosenfeld's conclusions concerning exhaustion have received considerable support from more recent work.

Coincident with these adrenocortical studies, the secretion of norepinephrine and epinephrine by the perfused calf adrenal was investigated in collaboration with a group from NIH (1339). The glands synthesized these catechol amines autonomously from dietary tyrosine, hydroxylation of tyrosine to DOPA was the rate limiting step, and norepinephrine was converted to epinephrine if a suitable methyl donor was provided. Catechol amine biosynthesis was strikingly reduced by anaerobic perfusion.

The last report from this productive program appeared in 1961 after Rosenfeld had left NMRI (1340). This reported the effects of altering electrolyte constituents of the perfusate on adrenal steroidogenesis. None of the ionic alterations except elevated potassium affected hydroxylase or hydrogenase activities. Increased potassium stimulated increased steroid secretion. However, omission of calcium, while having no immediate effects, eventually decreased corticoid secretion. Dinitrophenol increased adrenocortical metabolism, but depressed corticoid production by inhibiting the activity of steroid hydroxylases. Rosenfeld summarized in essence the results of this program with a scheme for adrenal steroidogenesis starting with simple carbohydrate precursors or cholesterol, including the enzymatic reactions and metabolic intermediates for converting cholesterol to pregnenolone and on to corticoids. It was suggested that ACTH acted to stimulate the conversion of glycogen to glucose-l-phosphate and not directly on the conversion of cholesterol to pregnenolone. This methodical and extensive program cleared away much of the mist shrouding the effects of irradiation on adrenocortical function and provided much valuable basic information on adrenocortical steroidogenesis.

In another study concerning the adrenal cortex Lt. Comdr. J. J. Martorano, MSC, USN, evaluated a series of environmental factors causing variations in levels of hepatic glycogen in adrenalectomized and intact mice (1035). This work was preliminary to subsequent studies on the relationship of electrolytes to liver glycogen.

We have seen how, beginning in 1947, research at NMRI reflected and contributed to a remarkable advance in knowledge of adrenocortical physiology in little over 10 years. We have also followed the research along unpredicted routes as new leads developed from successive experiments, as illustrated by the progression from adrenocortical hormone protection against certain noxious stimuli to the protection against alloxan diabetes conferred by epinephrine. One cannot avoid noting that behind much of this work was a conscientious effort to fulfill the Navy's desire to find protective measures against radiation injury in humans. Individual experiments by themselves may not appear to have been related to naval problems, but in viewing programs as a whole, the pertinence of these researches to the welfare of naval personnel is obvious. However, radiation is only one of many potentially injurious agents which may affect naval personnel under combat conditions; and research proceeded apace on the effects of other agents on adrenocortical function.

A marked increase in research on adrenocortical physiology occurred in 1952 with the arrival of Lt. D. H. Nelson, MC, USNR, and Lt. D. M. Hume, MC, USNR, and their active collaboration. Nelson joined the Endocrinology Branch early in the year and brought with him his techniques for measuring urinary and plasma corticoids. Somewhat later Hume joined the Circulatory Studies Group of Physiology and added his surgical skills and his knowledge of hypothalamic control of pituitary function to the collaborative effort. They first developed their classical method for chronic cannulation of the right lumbo-adrenal vein in dogs using a choker device around the adrenal vein to regu-

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late flow through the cannula so that blood samples could be collected without trauma at any time thereafter for analysis of corticoids from unanesthetized animals (804, 806). An improved assay for ACTH was developed based on this technique and the measurement of corticoid levels in the plasma from the catheterized lumbo-adrenal vein of hypophysectomized dogs (1180). The dogs could be used repeatedly and the method was useful in a range of 1 to 10 milliunits of ACTH. Corticoid production during operative trauma, in hemorrhagic shock, and in response to ACTH was also studied with the same techniques (804). Operative trauma increased corticoid secretion immediately, and independently of changes in blood flow through the adrenal, to levels much greater than those during convalescence. Despite reduced blood flow, hemorrhagic shock resulted in sustained high levels of corticoid secretion which fell only when blood flow approached zero. These same responses were observed in hypophysectomized dogs receiving continuous infusions of ACTH; so that there presumably were extra-pituitary factors enhancing corticoid secretion in these circumstances.

Lt. R. H. Egdahl, MC, USN, joined this group in September 1953 and an investigation was undertaken of the effects of hypothermia and cold exposure on adrenocortical function. These subjects were of interest to the Navy because of the potential usefulness of hypothermia in surgery and because of the threat of cold exposure to personnel in naval operations in the Arctic, Antarctic, and other cold climates.

Hypothermia produced a fall in 17-hydroxycorticoid secretion in anesthetized dogs (460) which was brought about by a marked decrease in secretion of ACTH, as well as an adrenocortical adrenal refractoriness to ACTH (454). The effect on the adrenal apparently was due to lowered temperature directly. On the other hand 17-hydroxycorticoid secretion remained at basal levels when unanesthetized dogs were exposed to −10° C. for from 1 to 33 hours. Dogs apparently do not respond to cold with increased adrenocortical function as do many other species, for example rats (1182).

Lt. J. B. Richards, MC, USNR, also well trained in procedures for measuring steroids, joined the Physiology Division and began collaboration with Nelson, Hume and Edgahl in July 1954. Nelson and Hume left NMRI in December 1954, but the program continued with Richards replacing Nelson and Egdahl continuing Hume's role.

One of the more bizarre aspects of this program was determination of corticoid secretion in a hypothermic black bear cub, a presumptive hibernator. Its principal corticoid was hydrocortisone and its secretion fell, as in dogs, with hypothermia (457). Egdahl and Richards (456) further investigated the effects of cold exposure on unanesthetized dogs. After an initial rise following exposure, hydroxycorticoid levels fell to normal within 1 to 3 hours and remained there during the balance of exposures at −46° to −50° C. for up to 28 hours and at −79° C. for up to 5 hours. Healthy dogs remained normothermic with these exposures and remained responsive to ACTH despite the return to normal levels of corticoid secretion. Surprisingly, two adrenalectomized dogs withstood equally well exposure to −46° to −50° C. for 4½ hours and one to −5° C. for 8 hours (463). In contrast, dogs subjected to hyperthermia had a rapid rise in rectal temperature and a twofold to sevenfold increase in corticoid output which was abolished by hypophysectomy (1310). Thus dogs responded in radically different fashion to hypothermia and hyperthermia, the latter having a marked stimulatory effect on corticoid secretion.

The effects of reserpine and chlorpromazine on adrenocortical function were also explored (455, 459). Both compounds, when given acutely, stimulated hydroxycorticoid secretion, especially at higher doses, and the response was abolished by hypophysectomy. However, all doses of reserpine exceeded minimal toxic levels. The only dose approaching a nontoxic level produced a minimal response. Chronic oral administration of nontoxic doses of reserpine inhibited pituitary adrenal function in singly caged and grouped mice (244). The effect was more pronounced on the latter. Other workers concluded later that reserpine acutely stimulated ACTH secretion, but chronically inhibited it. However, the differences in dosage with respect to toxicity probably were the most important factor accounting for the discrepancies.

The problem of maintaining atmospheric conditions within tolerable limits during prolonged submersion in submarines, or conceivably during space flight, prompted exploration of the effects of elevated ambient CO_2 levels and altered acid-base balance of the blood on adrenocortical secretion. Direct alteration of blood pH or CO_2-tension increased corticoid secretion markedly (1313). Exposure to elevated ambient CO_2 for 1 hour had a similar effect in anesthetized dogs. At 2.5 percent CO_2, 10 percent of the dogs responded with increased adrenocortical secretion and the numbers responding increased with increasing CO_2 levels (1311). Unanesthetized dogs responded to a more marked degree with three of five showing increased adrenocortical secretion of 2.5 percent CO_2 and all

dogs at levels of 5 percent or more of CO_2 (1314). The increased secretion rates were transient at the 2.5 and 5 percent levels of CO_2 and sustained at higher levels. Longer exposures to and increased concentrations of CO_2 produced proportionately greater secretion of steroids. Exposure to CO_2 produces respiratory acidosis which increases with increasing concentrations. Thus increases in ambient CO_2 stimulate increased adrenocortical secretion either directly, through acidosis, or both.

Hume and Nelson (807) participated in studying the personnel burned in the fire aboard the U.S.S. *Bennington* in 1954. They found that increased secretion of adrenal corticoids persisted for as long as 2 weeks after burning and then in uncomplicated cases, fell to normal values in blood and urine. Complications resulted in secondary rises in corticoid secretion. At no time was there evidence of adrenocortical failure or "exhaustion." These results indicate that the use of cortisone, ACTH, or both in burn cases is unwarranted and might be hazardous, especially as there was no indication of compromised pituitary-adrenocortical function.

"Stress" in military personnel under combat conditions is of direct interest to the Navy. Accordingly, the Navy participated in project "FAST" which was a joint military effort to evaluate "stress" in combat personnel during the Korean conflict. NMRI was represented by Comdr. D. Minard, MC, USN, who was responsible for endocrine and other physiological evaluations. A variety of measurements, largely indirect, were made to assess adrenocortical function. These indicated that under acute or prolonged combat stress there is adrenocortical stimulation, although there was wide variation between individuals (1129). Urines were collected for later determination of steroids by Pincus and Hoagland of the Worcester Foundation and Forsham of San Francisco.

J. J. Christian joined the staff of NMRI in 1951 and began research on the effects of crowding and changes in population density on pituitary-adrenocortical and reproductive function growth, survival and mechanisms of host resistance. This program was based on his hypothesis that density-dependent endocrine feedback systems controlled population growth and decline. D. E. Davis of the Johns Hopkins School of Hygiene and Public Health collaborated in parts of this work.

First it was demonstrated that adrenocortical weight increased and weights of the thymus, gonads, sex accessories, and body decreased as the number of male mice per cage increased. These changes were greater in aggressive brown house mice than in more docile albino mice suggesting a behavioral component in the genesis of the endocrine and somatic changes (240, 241). Similar changes occurred in response to increased numbers in similar experiments but with cages 80 times as large (247), thus indicating that the changes were a function of numbers and not of density per se. The endocrine responses were shown to be related to social rank (status) and therefore to social competition (376). Dominant animals were least and subordinate animals most affected. Fighting occurred when male mice were grouped, but injury from fighting did not contribute to the adrenal and reproductive responses to grouping (252). Although chronic treatment with reserpine suppressed pituitary-adrenocortical function, its effect on the responses to grouping were quantitatively far greater than on isolated mice, further implicating socio-psychological factors in the genesis of endocrine responses to increased numbers (244). Adrenocortical activity was not increased either inanition or by increased competition for food (250). However, inanition affected reproductive function adversely and independently of a generalized nonspecific pituitary-adrenocortical response. These experiments established that social competition ("social pressure") was solely responsible for the increases in adrenocortical and decreases in reproductive function associated with increases in population size.

When mice of both sexes were crowded together, diminished fertility and greatly increased intrauterine mortality occurred (245). Young born after removing the females from crowded conditions were stunted at weaning, but not at birth, as were their young in turn. The experimental design permitted assignment of this effect to inadequate nutrition during the period of nursing, probably due to inhibited lactation. The mechanism by which the young of the third generation were similarly affected is unknown. Thus crowding of females adversely affected at least two generations of their progeny.

It was hypothesized that an increase in adrenal corticoid secretion in response to crowding would inhibit inflammation and experimental granuloma formation, paralleling the effects of hydrocortisone injection. This hypothesis was confirmed (249). Along similar lines, Ellinger reported that grouping enhanced radiation mortality and that single-caging was essential for reproducibility of mortality curves (480).

The preceding experiments followed a basic plan in which young were singly caged at weaning, 4 weeks later placed together in groups of various sizes, and measurements made a week after grouping. This, while lending itself to adequate control, did not answer

the question of whether these same responses would occur to increasing numbers in freely-growing populations. Therefore, experiments were conducted in which a few pairs of mice were put in a large cage and allowed to reproduce at will. These populations were sacrificed at one or another point on their growth curves, usually after growth had ceased (248). The same endocrine and somatic responses to increased numbers occurred as in the preceding experiments except that the magnitude of the changes was greater. Adrenal weight increased in both sexes with evidence of increased adrenocortical function. Maturation of young and somatic growth was inhibited. Birth rates and survival rates of young were progressively depressed with increasing population size. Depressed birth rates were due to suppression of maturation, diminished fertility of adult females, increased intrauterine mortality, and probably in part to suppressed and abnormal spermatogenesis in males. Declining survival of young with increased "density" was probably due to diminished lactation. Increases in preputial weight in females greater than could be explained by ACTH were considered evidence of increased adrenal androgen secretion. The combined effect of these factors (in different proportions in different populations) was to slow and eventually stop population growth. Voles (*Microtus*) responded similarly to increases in population in the laboratory (247).

Finally it was shown in a series of experiments with feral Norway rats, voles (*Microtus*), and other species that these same responses to changes in population occurred under natural conditions (247). Artificially reducing the size of populations of rats resulted in a proportionate reduction in adrenal weight (cortical mass) (242) and adrenal weights reflected relative population size in general (243). These results were supported by the close relationship between changes in adrenal and pituitary weight and changes in a population of farm rats studied for a period of years (247). Increased social competition produced by adding aliens or substituting aliens for residents profoundly affected the growth of populations of rats (374). For example, substituting a large number of aliens for residents in a "high" population resulted in a striking decline from its original size.

In a long-term study of a vole (*Microtus*) population it was shown that adrenal weight was positively, and reproductive function in both sexes negatively, related to population size (247).

The results and conclusions from this program were summarized, in some cases expanded, in several reviews (247, 255, 257) including a discussion of the biological basis of rodent control (246); and a report on the role of density in populations of mammalian reservoirs of human disease (377). The whole field of endocrine adaptive mechanisms in the control of population growth was reviewed in detail in 1960 and included most of the work done at NMRI (253).

In 1958 Lt H. Varon, MC, USNR, on 2 years of duty in the Pathology Division of NMRI, began a collaborative study with Christian to determine the mechanisms involved in curtailing reproduction with increased population size, low social rank, or by other stressful stimuli, with particular attention to inhibition of sexual maturation. Evidence from a number of sources indicated that increased secretion of adrenal androgens following increased secretion of ACTH might inhibit production or release of pituitary gonadotrophins, especially in immature animals, hence suppress maturation. Physiologic levels of adrenal androgens were found to be capable of inhibiting the secretion of gonadotrophins (1665). This work was completed after both investigators left NMRI, but most of the experimentation was done at NMRI. Similarly experiments on crowding only females were carried out at NMRI with final completion elsewhere (254). Female mice responded to grouping with increased adrenocortical and decreased reproductive functions, but the adrenal responses were much less than in males under similar circumstances. Again brown house mice reacted to a greater degree than albinos There also was good evidence of increased adrenal androgen secretion in grouped females.

In 1955 a program was begun to assess the effects of high population levels on adrenocortical and reproductive functions and to explore the possible relationship of these to the pathogenesis of proliferative nonexudative glomerulonephritis in woodchucks. Glomerulonephritis was prevalent in a sample of these animals killed for exploratory purposes early in 1955, and it was this disease, coupled with ideal experimental conditions, that made a study of these animals interesting. This disease in one or another degree of severity was present in over 85 percent of the woodchucks (*Marmota*) on the Letterkenny Army Ordnance Depot. The renal disease was described in 1958 (256). In the course of this study, the reproductive cycle and history of woodchucks was described (1448).

This study was still in progress in 1960 and results, which will include those obtained with the support of NMRI, have appeared and will continue to appear over a period of years (259).

Endocrine changes associated with a sudden decline in a population of Japanese deer on James Island in

Chesapeake Bay were also studied under the aegis of NMRI (258). An abnormally high population of deer (300 on about 300 acres) collapsed in 1958 with a loss of 60 percent of the population in a few weeks. Moderately severe glomerulonephritis was associated with the high levels of population. Hepatitis appeared at the time of collapse and 2 years later was marked by the presence of cirrhosis in deer that were alive at the time of collapse. Greatly increased adrenocortical activity at the time of maximum density and subsequent mortality probably reduced resistance resulting in increased susceptibility to hepatitis. Neither hepatic nor renal disease accounted for the mass mortality. Following the reduction in population, somatic growth of young increased 40 percent, adrenal weight decreased from 40 to 60 percent and the renal disease underwent remission. Renal papillary PAS-positive granulation, gross symptoms, and histology of the adrenal cortex pointed to potassium deficiency, following prolonged adrenal hypercorticalism, as the putative cause of the mass mortality (260).

Thus between 1951 and 1960, pioneering experimental work at NMRI provided the major scaffolding for the structure of current concepts of population endocrinology and placed the concept of density-dependent behavioral-endocrinological feedback control of population growth and size on a solid factual basis. From this basis, numerous groups have begun further work in this field.

Besides the work on population endocrinology, several other investigations were carried out by Christian and his colleagues. The first of these was a publication on the relationship of adrenal weight to body size in a large number of species covering a great range in sizes (238). A study of reproduction, development, and natural history of a population of bats was completed (239). This included detailed histological descriptions of the ovaries and testes of young and adults throughout the year, an analysis of adrenal weight in relation to reproduction, and a description of toothwear and of annular rings of dentine in the teeth that could be used to determine age. Comdr. M. Wheatcroft, DC, USN, and Capt. J. English, DC, USN, of the Dental Division of NMRI assisted with the work on teeth. Finally, using data on breeding from the NMRI mouse colonies before and after the institution of a 15-hour daily light regimen, it was shown that a constant 15 hours of light per day resulted in a significant increase in the incidence of postpartum conception (1037).

In 1956 Lt. E. Steinberger, MC, USNR, joined the staff. He began a series of experiments on the effects of various agents on spermatogenesis in rats. It was found that triethylenemelamine (TEM) in low doses (0.05 mg/kg) for 30 days reduced fertility but produced no demonstrable morphological changes. However, a higher dose 0.2 mg/K for 5 days, also produced morphological changes resembling radiation damage with damage to spermatogonia and a maturation depletion of germinal elements (1478). These studies led directly to the later use of TEM to control natural populations of pests by D. E. Davis.

The damaging effects of heat on testicular germinal epithelium were studied using 15-minute controlled exposures (1479). There were no detectable morphological changes at 41° C., a general progressive destruction of all germinal elements starting with spermatids at 45° C., while at 43° C. primary spermatocytes were selectively destroyed. These experiments clarified the controversy on how heat damaged the germinal epithelium with the conclusion that heat produces a specific type of damage which is masked by general destruction of the germinal epithelium with excessive heat. Hence testicular damage due to heat differed from that produced by TEM or X-radiation.

Steinberger also carried out a series of studies on peritoneal absorption of fluids which have potentially great practical importance to the Navy in that they led to a simple method for rapid administration of fluids to injured personnel under field combat conditions. It was shown that hyaluronidase increased the rate of absorption of saline from the peritoneal cavity sufficiently to warrant its practical use (1477). It was further shown that phosphorylated hesperidin inhibited fluid absorption, probably by direct effects on permeability, and that this effect could be blocked by hyaluronidase. Adrenalectomy did not alter these effects. However, hyaluronidase increased the absorption of erythrocytes from the peritoneal cavity only slightly whereas phosphorylated hesperidin blocked it completely (1480).

Additional studies relating to reproductive endocrinology included demonstrations that spermatozoa could survive freeze-drying (1107) and that blood calcium and β-globulin levels of female pigeons rose markedly during egg-laying (1400). Other changes in plasma proteins of pigeons during the reproductive cycle also were described.

Miscellaneous endocrine studies were conducted by a number of other groups. Cortisone was found to increase mortality following placement of aortic grafts and to suppress the connective tissue component of the healing response (18). Thyroid pedicle grafts, free thyroid autografts, and L-tri-iodothyronine applied

locally in the ventricular myocardium of dogs with total heart block shifted the cardiac pacemaker to the area of implantation and temporarily increased the heart rate from the bradycardia of heart block. These experiments, although preliminary, have obvious implications relating to therapeutic management of heart-block cases (557). Finally, R. F. Steiner has concerned himself since 1952 with the physical chemistry of insulin, insulin conjugates, and thyroglobulin studies which are not strictly in the field of endocrinology but which have made use of protein hormone factors.

The Endocrinology Branch of the Division of Physiology ceased to exist early in 1957 following the departure of Vollmer from NMRI. What was formerly the Endocrinology Branch was incorporated into the Experimental Medicine Branch of the Physiology Division and endocrine research continued in this and other groups thereafter. However, work in this field was never confined to the Endocrinology Branch—personnel from other divisions and branches having made major contributions to endocrinology. Also it should be evident that the major research endeavor in endocrinology centered around the pituitary-adrenocortical and related systems, especially in terms of their responses to adverse environmental stimuli which might be encountered by naval personnel or in terms of their roles in the management of clinical problems.

Research in Endocrinology at NMRI has contributed importantly to the field and has involved a number of outstanding people in the field. In many instances NMRI provided investigators with freedom, opportunities, and facilities to carry out programs which would have been difficult, if not impossible, elsewhere. It would be difficult to overemphasize the importance that scientific freedom has had in the success of endocrine research at NMRI, although investigators were aware of naval problems and made every effort to channel their research in these directions. When one has this opportunity to look at past research in endocrinology at NMRI from a retrospective vantage point, the basic importance of the work done and its contribution to the understanding and management of problems arising from military operational or modern combat conditions becomes impressive. In addition, there have been major contributions to fields of biology and physiology as a whole.

X. MICROBIOLOGY

E. Weiss

During World War II scrub typhus was one of the more important, poorly comprehended infections encountered by the American forces in the Far East. The etiological agent, *Rickettsia tsutsugamushi,* was extensively studied at NMRI by McLimans et al (1080). Attempts were made to understand its pathogenesis by Tullis et al (1608) and to test potential chemotherapeutic agents by Steele et al (1473). This work was carried out before the broad-range antibiotics were discovered and the survey of chemotherapeutic agents yielded disappointing results, but a foundation of basic information was laid for the developments that followed.

Another health problem constantly confronting the Navy is shigellosis, or dysentery. In a well established community with an adequate sewage system and other sanitary safeguards, enteric infections occur infrequently. However, when a large number of sailors are crowded into a ship, or marines are sent to field exercises under difficult and stressful conditions, opportunities for breakdowns in sanitary barriers are considerable and outbreaks of dysentery often occur. The Naval Medical Research Institute has collected over the years the principal strains that have been responsible for the outbreaks in the Navy. This collection by Babcock et al (3) accompanied by the documentation of the outbreak that each strain produced, is one of the most complete in this country and is of great value for epidemiological studies. NMRI has been able to provide the American Type Culture Collection, universities, and other accredited laboratories with representative strains for teaching and research purposes. In this connection, the potential role of enteric infection was recently investigated in a crowded fallout shelter which was used to study conditions expected to prevail in passive defense against atomic attack. It was found that the fecal flora of the participants in the shelter project remained entirely normal and it was concluded that under the conditions of the test, dysentery can be prevented (J. A. Davies, unpublished).

The biochemical properties of enteric pathogens have been studied quite extensively by Erlandson (516, 518, 519), although the connection between biochemical characteristics and virulence has not been satisfactorily resolved for this or most of the other groups of microorganisms. To study the virulence of a microorganism it is necessary to reproduce the disease in some form in an experimental animal, something that is very difficult to do with the Shigella. A great deal of time and effort have been devoted to the search of a suitable experimental animal and some progress has been made in this direction. In some cases the virulence of a strain can be tested by injecting hens' eggs and by studying the survival of developing chick embryos (30, 517). The susceptibility to infection of mice was found by McGuire and Floyd (1070) to be increased by fasting and fatigue. In other cases the ligated segments of small intestines of rabbits and guinea pigs have been found to be satisfactory models for experimental dysentery (555).

Control of dysentery by means of vaccination is beset with two difficulties which have not yet been surmounted. Firstly, several antigenically different strains are involved in dysentery, and at best a vaccine can include only a few of the more common types. Secondly, most vaccines against single strains have proved to be ineffective, even when tested against the strains from which the vaccines were prepared. This is believed to be due to the fact that infection is confined to the enteric tract and is not readily exposed to the action of antibodies. The Naval Medical Research Institute has attempted to produce satisfactory

vaccines under the leadership of Barnes (24). These attempts as well as others carried out by other research groups have not been successful, although they have served to clarify the difficulty of the problem of control of dysentery.

Respiratory infections have received a considerable amount of attention at NMRI. Since epidemiological investigations have been extensively carried out at other naval laboratories, efforts have been concentrated on problems of host-parasite relationship. For example, Wagner and Bennet (1702, 1703) studied factors that influence and those that prevent the production of fever by influenza viruses in experimental animals. Somewhat similar studies, extended to include the effect of cortisone were conducted by Khoobyarian and Walker (903). It was clearly shown that cortisone, which is commonly used in the treatment of arthritis and other illnesses producing exaggerated inflammatory responses, actually reduced the resistance of experimental animals to the toxic effects of the virus. Similar results were obtained with an entirely different pathogen of man by Vollmer and Hurlbut (1682).

Another problem that is of considerable interest to the Navy is infection by viruses that are carried by mosquitoes and other arthropods, called arboviruses. Viruses of this group are very numerous and span the whole range of virulence from a low to a highly lethal level. Some, such as the virus of Eastern equine encephalomyelitis, transmitted by mosquitoes, occur in the United States and are responsible for isolated human infections and deaths. However, in this country the mosquito population is kept under surveillance and the various control programs usually keep it below the danger point. The military are acutely aware of the fact that when troops are sent to a foreign land, they may be exposed to larger and more heavily infected mosquito populations. Furthermore, the sudden increase in the number of susceptible individuals, represented by incoming troops, and other changes in population produced by an emergency, may result in catastrophic epidemics.

In the Far East two viruses are of paramount importance, Japanese encephalitis and dengue viruses. The first produces a disease which has a very high fatality rate, while the other, although not usually lethal, incapacitates patients for several weeks. Hurlbut has made a number of interesting observations on the transmission of Japanese encephalitis virus. He showed (817, 818) that the virus can multiply to very high titer in a mosquito, *Culex quinguefasciatus,* following parenteral inocculation and can be maintained in the mosquito by serial passage or by cyclic transmission by bite to the mouse and back to the mosquito. He also showed that the infected mosquito maintains the virus when held under conditions simulating hibernation (816) but does not transmit it to its offspring (808).

The ultimate goal of the other studies in this field has been to develop better methods for the isolation, growth, and identification of these viruses. Dengue virus is an especially difficult one to study, because it grows with some difficulty in mammalian cells and does not produce overt signs of host cell degeneration, which is the most convenient means of identifying the growth of a virus. Following basic observations by Banta (16) on the growth of several viruses in tissue culture, Wiebenga (1753) was able to maintain dengue virus by serial passage in a line of cells that had been derived from human skin. The virus produced minimal degenerative changes in the host cells, but its presence could be detected by the fluorescent antibody technique. This technique is based on a typical antigen-antibody reaction, except that the antibody is labeled with a fluorescent compound and, if retained by the antigen, a layer of infected cells attached to glass in this case, it can be detected by ultraviolet microscopy. By this mehod it could be ascertained that 10 percent of the cells were infected at any one time.

Since the cells were not destroyed by the virus, they could be cultivated indefinitely. After 15 months of continuous cultivation, they became virulent for uninfected human skin cells of the same line or for monkey kidney cells. Thus, a new strain of virus was derived, which, although identical to the original virus in every other respect, destroyed the host cells and was a much more useful tool for laboratory investigations.

Hurlbut, in a more recent study (819), followed an entirely different approach to the study of arboviruses. He selected 13 representative strains and injected them, with a special microsyringe and needle that he devised, into 1 arachnid and 8 insect species representing 6 orders, a total of 117 combinations. He found that the viruses had a high potential for propagating in a variety of arthropods, but that not all viruses multiplied to the same extent. He proposed a theory that the ancestral virus type had a wide host range, while the more recent evolutionary types were restricted to few hosts. On the basis of his theory he traced the evolution of the arboviruses that he had tested. Furthermore, he demonstrated that the viruses were duplicated without any apparent injury to the host, but had an affinity for the central nervous system.

These results are of great significance, both from the point of view of basic information and implications

regarding possible health hazards. It is obvious from these studies that the viruses can multiply in a larger number of arthropods than heretofore believed possible. Their occurrence in just a few of these hosts in nature is probably due to a number of ill-defined factors and circumstances that are not all difficult to overcome. It is conceivable that under certain emergency conditions that affect the ecology of the arthropods and the animals on which they feed, a new vector for a virus could emerge, that would precipitate an entirely new health hazard.

A problem of an entirely different nature that is of great interest to the military is epidemic typhus. This disease has ravaged armies and civilian populations during all major wars for millennia. The possibility that it may reemerge as a devastating force in any future conflict cannot be discounted, especially if large segments of the population are exposed to ionizing radiation. It was shown over 30 years ago that X-irradiated rats are far more susceptible than normal animals to infection with the agent of epidemic typhus. This phenomenon was also recently studied at NMRI. It was shown by Weiss and Dressler (1730) that tissue culture cells exposed to very large doses of gamma radiation supported the growth of the microorganisms—called rickettsiae—just as well as normal cells, but released them more rapidly. If this is an indication of what happens in the irradiated animals, it can be readily seen that more rapid release of rickettsiae leads to more rapid infection of new cells and greater pathogenicity.

Unlike the previously discussed arboviruses, the vector of the agent of epidemic typhus, the body louse, can be controlled by DDT and other insecticides, and, unlike the Shigella, a valuable vaccine is available. Furthermore, if the patients are treated promptly, the broad range antibiotics are effective. However, it would be a mistake to believe that DDT, the vaccine, and the antibiotics present an insurmountable barrier to the progress of an epidemic. Strains of insects resistant to DDT are known to occur, the vaccine does not afford complete protection, and wide use of antibiotics may accelerate the appearance of resistant strains. The latter problem has been studied extensively at NMRI. It was shown by Weiss and collaborators that, fortunately, it is relatively difficult to produce antibiotic-resistant strains in the laboratory. For example, strains resistant to the tetracycline compounds were not produced, despite elaborate attempts. With chloramphenicol only two small steps in resistance could be obtained (1738). On the other hand, susceptibility to erythromycin, one of the most effective antibiotics, can be completely lost in a single step (1733). Rickettsiae have the relatively uncommon property of being inhibited by the sulfonamide antagonist, para-aminobenzoic acid. By producing several strains resistant to this drug, several properties of the rickettsiae became apparent. For example, it was shown that folic acid does not play the same role in rickettsiae as in many other microorganisms (1729, 1731, 1734). This work was reviewed in detail by Weiss in a recent publication (1735).

Rickettsiae, although bacteriumlike in most respects, have not yet grown in a cell-free medium, but because of their rather wide enzymatic capabilities, it is believed that it should eventually be possible to find a cell-free medium in which they can be grown. Much of the research at NMRI has been directed towards this goal, which would open new avenues for the production of antigens and vaccines. The direct approach—testing complex media of various kind—has not been fruitful and has not been extensively applied. Instead, attempts have been made to detect additional enzyme systems that may eventually lead to the determination of growth requirements. The most successful investigation has been concerned with a rickettsialike microorganism isolated at NMRI by Suitor (1551), which serves as an excellent model for "obligate" intracellular microorganisms. By the use of radioactive compounds it was found that this microorganism utilizes several carbohydrates, amino acids, and lipids for energy as well as synthesis (1198, 1199, 1740, 1741). The very active lipid metabolism of this agent is of particular interest, because it had not been previously described in microorganisms of this type. It appears logical that intracellular microorganisms such as rickettsiae should have an active lipid metabolism because their lipoproteic membranes must be very active in discerning and taking in labile nutrilites that might become fleetingly available within the cells.

The microorganisms of the psittacosis-trachoma group like rickettsiae, although not viruses, are other examples of obligate intracellular parasites which have been investigated at NMRI. The properties of the agent of psittacosis, reviewed by Weiss (1727) make it a somewhat more useful microorganism for basic investigation than trachoma. One of the problems that has confronted investigators of this aspect of microbiology is the great variety of strains related to the agent of psittacosis that are continuously encountered in nature. The mechanism by which these strains arise is puzzling. It has been shown by previous investigators that changes in virulence for experimental animals can be produced by serial passages in the guinea pig,

mouse, or chick embryo. It has also been shown that resistance to sulfonamides is acquired readily when the agent is grown in the presence of sulfadiazine. Gordon et al had previously elsewhere developed strains resistant to penicillin and chlortetracycline. These strains were then used (651, 653) in mixed cultures to investigate the possibility of interaction. It was clearly shown that the strains do interact among each other. For example, when a strain resistant to chlortetracycline and one resistant to sulfadiazine are grown together, a strain with dual drug resistance can be rapidly obtained. The same results are obtained when one of the two strains is subjected to heat sufficient to destroy the infectivity, but not high enough to break up the nucleic acid.

The above described results are of paramount importance. They indicate that the agent of psittacosis, in addition to having a rather high rate of mutation, can exchange genetic information with related strains. The role of this genetic exchange in the establishment of new strains in nature is not known, but conceivably is an important one, especially since full activity, including infectivity of both strains of microorganisms is not required.

Trachoma, the disease, constitutes a very important problem. It is estimated that it affects approximately 400 million people throughout the world. It presents no great danger to the military entering an area where the disease is prevalent and, because of the chronic nature of the infection, it is not likely that an emergency situation would spark a new epidemic. The Navy's chief interest in trachoma rests on the geographical location of its two oversea laboratories, Naval Medical Research Units 2 and 3, in Taiwan, Republic of China, and Cairo, U.A.R., and the opportunities it has to study this problem and, possibly, offer suggestions to alleviate this burden on the affected populations.

For the past 6 years, it has been possible to grow this agent in chick embryos, and this has given new impetus to investigations of various kinds such as surveys of strains from various areas of the world, more rational approaches to treatment, and possibly, vaccination. This information was well summarized at a symposium on trachoma, which was sponsored by the New York Academy of Sciences (657) in 1961 and was organized by Gordon. The Department of Microbiology of NMRI, under his leadership, has been able to contribute a great deal to the broad program of research on trachoma. This was in part due to the wide experience that he and his associates had acquired with the related agent of psittacosis. Procedures for the laboratory diagnosis of trachoma were greatly simplified. Tissue culture techniques developed for other agents by Weiss and Dressler (1734) were adapted to trachoma by Gordon (655) and a very simple rapid staining method of the typical inclusion bodies by iodine was introduced (659). An investigation of the drug susceptibility of the agent (657) greatly facilitated the selection of drugs to be used in isolation attempts. A recent development has been the demonstration that increasing the level of glucose in certain tissue culture media greatly favors the growth of the agent. Thus, through these efforts, techniques for the direction of active trachoma infection, once laborious and difficult, have become simplified to the extent that they can be applied extensively. For example, Gordon et al (660), by applying these techniques, were able to follow the course of experimental trachoma in monkeys. Conjunctival swabs and washings were taken repeatedly and tested for the presence of viable trachoma agents. Such determinations now require only 2 days instead of weeks. Valuable observations could therefore be made on the correlation between overt signs of disease and presence of cultivable microorganisms. The Department is now engaged in a detailed antigenic analysis of the various strains of trachoma, which is essential for an understanding of the nature of immunity to this infection and opportunity for vaccination.

The approaches to the above described problems, being investigated at NMRI have been basic, since other laboratories are in a better geographical position to carry out surveys and apply findings to specific problems. No attempt is made to cover all phases of research of interest to the Navy. Instead, NMRI is making an effort to become a center of study for a few important problems which are of particular interest to the Navy, are generally neglected by other agencies, but are of basic interest to the scientific public at large.

XI. PARASITOLOGY

C. G. Huff

World War II Period

Parasitological research at NMRI began as a congerie of unrelated projects necessitated by the exigencies of World War II. The world conflict into which the United States was so precipitously thrown involved campaigns in parts of the world where parasitic diseases were rife. In some instances such as the invasion of Guadalcanal by the U.S. Marines, parasitic diseases—in this case, malaria—proved to be more formidable foes than enemy troops. Consequently the parasitological research done at NMRI during the war consisted of "crash" programs. Research in antimalarial drugs was initiated by studies on the effects of the gas, stibene, upon a newly discovered malarial parasite (*Plasmodium gallinaceum*) which infects chickens (1473). This work was rapidly expanded into the testing of a wide variety of compounds in an effort to find a substitute for quinine, the supply of which had been cut off by Japanese conquest (1543, 1544, 1546, 1565). Another approach toward insect-transmitted diseases was a program aimed at developing better repellents against insects, which could be applied to the skin or clothing of troops (504, 843, 844, 1045, 1263, 1264, 1265, 1765). Closely related was the attempt to control diseases caused by blood flukes (schistosomiases) through chemical attacks against snails in which the immature stages of these worms spend a part of their life cycles (841, 940). Another disease which is more serious in the tropics than in temperate regions is amoebic dysentery. Since many of the methods of water purification are not effective against the transmissible stage of this parasite, methods were sought for rendering drinking water free from the cysts (1040, 1041, 1042, 1043, 1542). Other scientists associated with these wartime programs were: R. E. Kuntz, J. H. Killough, C. S. Wilson, D. R. Mathieson, M. A. Stirewalt, L. A. Jachowski, and K. L. Knight. There was a transitional period following the war in which some of the wartime research projects were completed, some military personnel left NMRI, and civil service scientists were recruited. In 1947 C. G. Huff was appointed head of the parasitological laboratories and research began to be oriented toward long-term basic investigations.

Postwar Period

Although interest in parasitic diseases had been greatly heightened by the war and research had been greatly expanded in universities and commercial laboratories as well as in Government laboratories, it was realized that the peacetime continuation of these investigations would become largely the responsibility of Government agencies such as the Public Health Service and the Department of Defense. Now interest and support of parasitological research in non-Government institutions have fallen to about their prewar level. Parasitic diseases in continental United States have either been eliminated (malaria), or have been reduced to such low levels as no longer to enlist interest and research. In spite of this fortunate situation in the United States, parasitic diseases still remain of very great importance in many other parts of the world, particularly in the tropics. These diseases not only take their yearly toll of lives but remain formidable obstacles in the economic, social, and educational development of the people in the countries in which they exist. Furthermore, they present potential problems to our military forces in the event of possible engagements in these countries.

Since basic information on parasitic diseases—in spite of all of the great developments during the past 20 years—is woefully deficient, it is quite apparent that research aimed at increasing this information must be carried out in such laboratories as the Naval Medical Research Institute where it can be given the continuity so essential to its success. Such basic studies have been pursued at NMRI for the past 15 years on malaria, schistosomiasis, filariasis, and insect vectors of disease.

Malaria.—The research work in malaria has covered a wide scope. The diverse problems which have been studied can be grouped in two categories: those concerning the relationships between the malarial parasites and their vertebrate hosts, and those concerning the relationships between the malarial parasites and their mosquito hosts.

In the former category most of the research has dealt with the stage of the parasite which resides in fixed tissue cells (exoerythrocytic stages) rather than in the red blood cells. Using the conventional methods employed in the study of histological preparations the morphology, development and localizations of these stages of seven species of avian malarial parasites were studied in nine species of birds (774, 780). In addition to wide differences in the ability of these stages to infect different species of birds definite patterns of their development were observed in certain host-parasite combinations (782, 792). Some of these were closely related to the course of development of parasites in the red cells (parasitemia). For example, in two of the species which infect turkeys a massive invasion of the endothelial lining cells of the blood vessels occurred very regularly at 13 to 15 days after the inoculation. At that time the infection of the blood would have subsided but the animals would invariably die as a result of this attack on the cells lining the blood vessels of the vital organs.

Three species of parasite were adapted to continuous passage in chick embryos. Tissue cultures were prepared from the infected organs of the embryos and by designing special chambers it was possible to observe and photograph the fixed tissue cells in all stages of development. The host cells and parasites which are barely visible by the ordinary bright field microscope were observed in great detail by means of phase contrast optics which had been introduced shortly before World War II. By the use of normal speed and time-lapse moving pictures, the behavior of the living fixed tissue stages was observed. In addition, the interaction of parasite and host cell could for the first time be studied in the living condition (795). These methods have opened up for investigation a very large number of problems concerning the fixed tissue stages which could formerly not be studied (796).

Parasitologists are interested in the effects of the hosts upon the parasite as well as the effects of the parasite on the host. One such effect which was given considerable study concerned the effect of the avian host upon the sexual stages of the parasites (gametocytes) which complete their development in mosquitoes. It has been known for some time that as the infection develops in the host the ability of the sexual stages of the parasite in the blood to produce infections decreased in mosquitoes which ingested this blood. Many possible factors which might be considered as possible causes of this effect were studied. The only factor which was shown consistently to have such an effect on these sexual stages was the development of active immunity in the avian host (785, 793).

Among the many studies made of the interrelationships between malarial parasites and their mosquito hosts two principal programs will be mentioned. First, an extended program of investigations (still in progress) has been directed toward the effects of antimalarial drugs, antibiotics, bases, acids, and salts upon the susceptibility of mosquitoes to malaria. A vast array of effects has been found which has led to the general conclusion that natural immunity and susceptibility of mosquitoes to malarial infection are to a large extent determined by the physiological status of the mosquito (1567, 1575). The second program was concerned with the potentialities of various stages of the malarial parasites to develop in the body cavity of mosquitoes and to produce infective forms in their salivary glands. Previously it had been believed that the known complex course of development and migration of the parasite in the body of the mosquito was the only possible course. It has now been proved that the sexual stages normally ingested in the blood meal of the mosquitoes can be injected into the body cavity and that their development may occur in all parts of the body (instead of, as formerly believed, upon the stomach only), and that the forms infective to the vertebrate host (sporozoites) will develop in the salivary glands of the mosquito in the usual fashion. Furthermore, any of the intermediate stages between the sexual stages and the sporozoite can be removed from an infected mosquito, transferred to the body cavity of another mosquito, and will continue the remainder of their development in the recipient mosquito. Also, the fixed tissue stages grown in tissue cultures can be injected into the body cavity of mosquitoes and they will produce infective forms in the salivary glands. These

surprising results have thrown much light upon the mechanisms which determine whether a particular mosquito will become infected with a particular kind of parasite (1717, 1719).

Schistosomiasis.—A group of parasites of potentially great importance to the military forces is the group known as schistosomes or blood flukes. The diseases, variously known as genitourinary schistosomiasis or bilharzia, Manson's schistosomiasis, and Katayama disease or Oriental schistosomiasis, are alike in that the parasites have their early development in snails, infect man by direct penetration of the skin when exposed to infested waters, and reinfect snails through the egg stage voided by infected persons. They differ widely in their pathology and symptomatology. A broad program of studies began with a search for the best species of animals available for maintaining them in the laboratory (1523) and has continued in the biological immunological, biochemical, and physiology phases of the disease in these animals (1522, 1527). Much of this study has been directed toward the free-swimming stage (cercariae) which penetrates the skin of the host. The mechanisms by which these infective stages invade the skin of the vertebrate and the pattern of their behavior after invasion have been intensively investigated (1531, 1534). Likewise, the question of how resistance to subsequent penetration of the skin develops in hosts which have been recently exposed to attacks by the infective stages has been analyzed (1533). Infective stages (cercariae) which have been placed in contact with the serum of the infected host form a thick envelope around them (937). The antibody responsible for this reaction has been located by electrophoresis and its characteristics determined (530). The secretions from two different types of glands in the cercariae have been collected and their general chemical nature determined (1534, 1536, 1537). It was demonstrated that one species of snail is capable of sustaining the development of the schistosomes only if the environmental temperature is maintained above 25° C. Improvements in the laboratory diagnosis of schistosomiasis have been made (861).

Filariasis.—Another group of diseases caused by worms are the filariases. They are tropical in distribution, are transmitted by mosquitoes, and are of potential importance to military operations where they occur. Bancroft's filariasis may exhibit elephantiasis as one of its symptoms. A long program of research on this disease has been carried out at NMRI and on missions to American Samoa (851, 856) and Puerto Rico (863). Epidemiological investigations in Samoa demonstrated that this infection is transmitted primarily outside of villages in wooded areas. It was found that a good way of controlling the disease was by aerial spraying of insecticides which destroy the mosquitoes responsible for transmitting it from man-to-man. The drug used in treating human infections was found to act primarily on the larvae of the worms which circulate in the blood. The species of mosquito most important in transmitting the disease in Samoa (*Aedes polynesiensis*) was given extensive field and laboratory study which provided information on survival, breeding, feeding habits, and flight range. This information has been useful in control programs directed toward reduction of the mosquito population. Laboratory and field work in Puerto Rico have contributed information on the relation of filariasis to the occurrence of tropical hydrocele and to the serodiagnosis of filariasis.

Insect vectors of disease.—In recent years and currently, considerable emphasis has been placed upon two aspects of research on mosquitoes, namely: (1) physiology of digestion and (2) behavioral studies and response to radiation. It was discovered that the digestion of blood in two species of mosquitoes was significantly affected by the feeding of cations, antibiotics, and metabolites, and that these substances exhibited highly complex binding relations among themselves (1579, 1580). The amino acid makeup of excretions has been determined (829). Some of the factors which have been shown to affect the biological characteristics of one of the malaria-carrying mosquitoes (*Anopheles quadrimaculatus*) are: (1) density of larval population (which influence the mating habits, biting rate, and longevity of adults) (1566); (2) population age and sex ratio (which influence the biting rate); (3) light; (4) size of container; and (5) length of adaption time in the laboratory [which influences the biting habits and survival of the adults (1466, 1574)]. It has been shown that the lethal effects of gamma radiation on another species of mosquito (*Aedes aegypti*) may be modified by permitting the mosquito to take a series of blood meals (1576, 1582). A strain of this species, somewhat more resistant to gamma radiation, has been obtained by selection over a period of 28 generations.

XII. DENTISTRY

H. W. Lyon

Prior to 1942, research in naval dentistry was centered and directed mainly from the Naval Dental School, although some investigations were carried on independently at other activities and sometimes at the individual's own expense. Facilities for most laboratory and animal studies were not available. With the commissioning of the Naval Medical Research Institute the first full-time dental research facility was established.

Dental research at NMRI has historically been integrated with work done at the Naval Dental School, as well as the National Bureau of Standards, the American Dental Association, the National Institute of Dental Research, and Georgetown University. Recently, collaborative studies with the Armed Forces Radiobiology Research Institute have been initiated.

Dental Research During World War II

During World War II, the Navy Dental Department conducted emergency studies of direct military application. New studies were initiated involving manpower logistics from which the needs of the Dental Department, and the dental status of naval personnel in this period of mobilization could be estimated. The dental records of approximately 70,000 Navy volunteers were analyzed and evaluated. Several reports indicated first, that 12,000 dental officers were needed per year to restore and maintain oral health of 3,000,000 Navy inductees (1308); second, that regional variations in the oral health affected the needs of the area (1367, 1368); and third, that age and regional variations affected the degree of prosthetic requirements (1369).

In addition to these statistical studies, the rapid advances in the technology of modern warfare presented other acute problems for dentistry. In 1943–44 as man flew higher and faster, pilots reported severe, excruciating toothaches due to rapid changes in barometric intrapulpal pressure. The results of certain experiments using simulated altitude chambers in this division led to new methods in restorative dentistry for protection of the sensitive dental pulp (1305).

Use of localized refrigeration anesthesia procedures showed that cooling of the alveolar process from 37° to 12° C. provided adequate protection from pain caused by operative dentistry procedures. No damage to either hard or soft tissues was discernible (1309).

Concurrent studies were also in progress regarding the effect of total body vibration on the dental pulp and supporting tissues of the teeth. Histological evaluation showed no evidence of morphological change (1306). It does seem significant that these findings proved of value some 20 years later in relation to the vibratory effect of takeoff and reentry as experienced by the astronauts in the current Mercury program.

Postwar Studies

Experimental dental caries.—The experiences of World War II showed that the replacement of lost tooth structure due to dental caries was still the most important problem facing the Navy Dental Corps. The need for basic research in preventive dentistry led to the initiation of a breeding program to produce from the standard Osborne-Mendel strain, a rat strain which would be uniformly susceptible to dental decay, but otherwise, possess the characteristics of the normal laboratory rat (1373). This strain, the NMRI–D rat, produces clinically visible smooth surface or pit and

fissure caries in 30 days on a cariogenic diet (985). Other standard strains generally require 80 to 100 days to produce smooth surface or pit and fissure cavities, but not both. The economy in research time is obvious and the significance of the simultaneous occurrence of both types of caries has not yet been solved. Establishment of the strain has permitted many studies concerning the epidemiology, etiology, and treatment of dental caries; numerous activities have requested breeding stock. One of the first group of studies showed that some popular acid beverages possess a sufficiently low pH to decalcify the enamel of teeth (1067). The major effort was slanted at an evaluation of the influence of dietary elements on dental caries. A basic purified diet that satisfies the strain's nutritional requirements permitting experimental variations in components has been established (1373). Growth patterns and bilateral caries symmetry have been evaluated early in the study (1745). Repeated experiments have shown that NMRI–D strain rat is less susceptible to the caries reducing effect reported by others, e.g. sugar free diets (996), phosphates (1660), or molybdenum (1661). These experiments demonstrate strain differences in caries activity and illustrate the danger of extrapolating from experimental animals to human application. On the other hand, this same applied research experience provides capability for investigating factors of dental interest in the space age when personnel may travel for extended periods of time in closed vehicles, provided only with concentrated diets and distilled water.

Effects of ionizing radiation on oral tissues.—Certain oral manifestations of total body irradiation as seen in Nagasaki and Hiroshima patients were the presence of acute fulminating necrotizing gingivitis plus ulceration of the buccal mucosa. Collaborative studies with the Hematology Division, NMRI, showed that in total body X-ray irradiated dogs, ulcerative gingivitis developed during the early stage of hemopoietic depression, reaching the fulminating stage as the animals became moribund (1187).

The Bikini test trials and associated total body X-irradiation studies on swine presented an opportunity to observe the effects of high energy irradiation (507). These studies showed that the ameloblast was especially susceptible to injury, having a pronounced effect on the developing tooth. Hemorrhage within the follicular sac was a common observation.

The effects of bilaterally applied X-ray irradiation to the head and neck of dogs, in doses ranging from 1000 to 1750 roentgens, yielded further valuable information (511). Salivary gland parenchyma showed evidence of severe injury, followed by bizarre changes in glandular cell architecture. Cell damage proved to be irreversible at higher dose levels. Dosimetry measurements indicated summation at the mid-line (1746), from bilateral application in equal dose rates.

The metabolism of exteriorized salivary glands in the rat was affected by X-ray irradiation, these changes being especially noticeable in various enzyme systems (513).

Field studies at Frenchman's Flats revealed that many dental materials became dangerously radioactive after capture of thermal neutrons when released by nuclear explosions (828). Studies are now in progress regarding the effect of thermal and fast neutrons on oral tissues and dental restorations.

Development of cross-species bone grafting procedures.—Anorganic bone, or bone treated with ethylenediamine for removal of the nitrogenous organic matrix resulted as a serendipitous outgrowth from studies concerned with an evaluation of the chemical components of normal and abnormal teeth and bone (987). Removal of the organic matrix results in complete despeciation, hence any antigenic response from cross-species implant procedures is precluded. In subsequent applied research, anorganic bone was found to be an acceptable implant material for the restoration of osseous defects in maxillo-facial and orthopedic sites (184, 823). Ironically, this bone, though developed in the Dental Division, is remodeled and reconstructed more rapidly in orthopedic sites than in maxillo-facial areas. Although anorganic bone is remodeled at a slower rate than conventional autologous and homologous materials, its unlimited source, infinite shelf life and inexpensive preparation properties make this a useful substance if needed in a national emergency.

Response of pulpal tissues to operative dentistry procedures.—The introduction of high-speed cavity preparation techniques through use of the air turbine handpiece (250,000 r.p.m.) was a radical change from conventional methods of restoring teeth. In this procedure, a stream or spray of room temperature water is used to cool the bur and also serves as a lavage by flooding the cut surfaces of enamel or dentin. It was shown in this laboratory that in this technique, the dentinal tubules become extremely permeable, thus allowing penetration to the pulp by many agents placed within the tooth. Results of this experiment indicated that a cavity liner or sealant is a prerequisite before placing the permanent restoration.

Biochemistry studies concerning organic components of bone, teeth, and saliva.—Previous studies have shown that the natural luminescent qualities of the organic matrix of tooth structure are affected by dental caries, pulpal vitality, and by the inherent protein makeup of the collagen molecule. Current studies are exploring the fluorescence properties of normal and abnormal teeth and bones. Decalcified bone, dentin, and enamel were acid hydrolyzed to di- and tri-peptides. After removal of residual acids on Dowex columns, the peptides were submitted to starch gel electrophoresis. Three separate zones fluoresced, two being associated with resin artifacts and one cationic fraction as true gelatin associated. Identification of this fraction is not being pursued.

Associated with these studies is the development of a "tooth fluorometer." The clinical significance of these efforts can be stated quite simply: Fluorescence studies may provide the clinician with a new diagnostic tool for use in vitality studies and prognoses.

Regarding certain organic components of saliva, interests are focused on the significance of sialic acid and other associated protein substituents in patients with and without gingival disease (1023). Sialic acid, produced by the sublingual and submandibular salivary glands, appears to have a protective or preventive function associated with the maintenance of gingival health. Separation of these protein substituents is being evaluated through techniques of protein fractionation, spectroscopy and osmetry (761). Recent studies have shown that acute gingival disease is a manifestation observed in patients undergoing severe conditions of stress.

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